FILE 'USPATFULL, USPAT2, CAPLUS, WPIDS, IFIPAT, DGENE, PROMT, EMBASE, NLDB, SCISEARCH, DRUGU, BIOTECHNO, TOXCENTER, PASCAL, MEDLINE, PHIN, BIOSIS, JICST-EPLUS, IPA, BIOTECHDS, CANCERLIT, BIOBUSINESS' ENTERED AT 13:49:47 ON 14 JAN 2005
L3 18793 S (SLOW OR SUSTAINED)(W) RELEASE AND (PEPTIDE OR POLYPEPTIDE) AND POLYMER
SET PLURALS ON PERM
SET ABBR ON PERM
L4 16905 DUP REM L3 (1888 DUPLICATES REMOVED)

FILE 'REGISTRY' ENTERED AT 14:04:12 ON 14 JAN 2005
L5 1 S EXENDIN/CN
E L3
E EXENDIN
L6 0 S EXENDIN-3/CN
EXPAND EXENDIN-3 CN
L7 2 S E4 OR E5

FILE 'USPATFULL, USPAT2, CAPLUS, WPIDS, IFIPAT, DGENE, PROMT, EMBASE, NLDB, SCISEARCH, DRUGU, BIOTECHNO, TOXCENTER, PASCAL, MEDLINE, PHIN, BIOSIS, JICST-EPLUS, IPA, BIOTECHDS, CANCERLIT' ENTERED AT 14:10:35 ON 14 JAN 2005
L8 160 S L7

FILE 'USPATFULL, USPAT2, CAPLUS, WPIDS, IFIPAT, DGENE, PROMT, EMBASE, NLDB, SCISEARCH, DRUGU, BIOTECHNO, TOXCENTER, PASCAL, MEDLINE, PHIN, BIOSIS, JICST-EPLUS, IPA, BIOTECHDS, CANCERLIT' ENTERED AT 14:14:28 ON 14 JAN 2005

FILE 'BIOTECHNO, TOXCENTER, PASCAL, MEDLINE, PHIN, BIOSIS, JICST-EPLUS, IPA, BIOTECHDS, CANCERLIT' ENTERED AT 14:23:23 ON 14 JAN 2005
L9 57 S L7
L10 52 DUP REM L9 (5 DUPLICATES REMOVED)
L11 1 S L10 AND (SLOW OR SUSTAINED) (W) RELEASE
L12 45653 S (SLOW OR SUSTAINED) (W) RELEASE
L13 35 S (SLOW OR SUSTAINED) (W) RELEASE AND (POLYPEPTIDE OR PEPTIDE) AND (SUCROSE OR TREHALOSE OR MANNITOL)
L14 31 DUP REM L13 (4 DUPLICATES REMOVED)

=> d l11 ti abs

L11 ANSWER 1 OF 1 TOXCENTER COPYRIGHT 2005 ACS on STN
TI Exenatide: AC 2993, AC002993, AC2993A, exendin 4, LY2148568
AN 2004:55773 TOXCENTER
CP Copyright 2005 ACS
AB A review. Exenatide [AC002993, AC2993A, AC 2993, LY2148568, exendin 4], a glucagon-like peptide-1 (GLP-1) agonist, is a synthetic exendin 4 compound under development with Amylin Pharmaceuticals for the treatment of type 2 diabetes. Both exendin 4 and its analog, exendin 3, are 39-amino acid peptides isolated from Heloderma horridum lizard venom that have different amino acids at positions 2 and 3, resp. Exendins are able to stimulate insulin secretion in response to rising blood glucose levels, and modulate gastric emptying to slow the entry of ingested sugars into the bloodstream. Amylin Pharmaceuticals acquired exclusive patent rights for the two exendin compds. (exendin 3 and exendin 4) from the originator,
Dr John Eng (Bronx, NY, US). On 20 Sept. 2002, Amylin and Eli Lilly signed a collaborative agreement for the development and commercialization of exenatide for type 2 diabetes. Under the terms of the agreement, Eli Lilly has paid Amylin a licensing fee of $80 million and bought Amylin's stock worth $30 million at $18.69 a share. After the initial payment, Eli Lilly will pay Amylin up to $85 million upon reaching certain milestones and also make an addnl. payment of up to $130
million upon global commercialization of exenatide. Both companies will share the US development and commercialization costs, while Eli Lilly will pick up to 80% of development costs and all commercialization costs outside the US. Amylin and

Eli Lilly will equally share profit from sales in the US, while Eli Lilly will get 80% of the profit outside the US and Amylin will get the rest. This agreement has also enabled Amylin to train its sales force to co-promote Lilly's human growth hormone Humatrope. Alkermes will receive research and development funding and milestone payments, and also a combination of royalty payments and manufacturing fees based on product sales. Alkermes undertakes the responsibility for the development of several initial formulations of the long-acting drug and manufacturing of the final product, while Amylin will be responsible for clin. trials, regulatory filings and worldwide marketing. The goal of the exenatide LAR program is to develop a once-a-month injectable formulation of exenatide. In Nov. 2003, Amylin announced pos. results from the second of three pivotal, phase III studies that evaluated the effects of exenatide in combination with sulfonylureas in 377 randomized patients with type 2 diabetes. The design of the study was similar to that from the first study. The final third phase III study of exenatide was completed in Nov. 2003. This study investigated the effects of exenatide in combination with metformin and sulfonylureas. Amylin and Eli Lilly announced that all of the pivotal phase III trials met the primary glucose control endpoint as measured by glycosylated Hb. An NDA submission for exenatide is projected for mid-2004. A phase II, dose-ascending study in patients with type 2 diabetes was initiated in June 2002. This multicenter (US), double-blind, placebo-controlled study evaluated the safety, tolerability and the pharmacokinetic profile of exenatide LAR in up to 100 patients with type 2 diabetes. A phase I study of exenatide LAR began in Europe in Mar. 2001 and was completed in Q3 2001. A long-acting, **sustained-release** formulation of exenatide lowered both pre- and post-meal glucose concentration during a 24h period in patients with type 2 diabetes. In Nov. 2002, analysts at Prudential Financial estimated that exenatide, pending approval, has the potential to reach sales of $US477 million in 2006.

=> d l14 ibib ti abs 1-31

L14 ANSWER 1 OF 31 PHIN COPYRIGHT 2005 PJB on STN

ACCESSION NUMBER: 2004:7501 PHIN
DOCUMENT NUMBER: W00839778
DATA ENTRY DATE: 1 May 2004
TITLE: March Patent Applications
SOURCE: Target (2004) No. 29 p10
DOCUMENT TYPE: Newsletter
FILE SEGMENT: FULL
TI March Patent Applications

L14 ANSWER 2 OF 31 TOXCENTER COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2004:271229 TOXCENTER
COPYRIGHT: Copyright 2005 ACS
DOCUMENT NUMBER: CA142010115549N
TITLE: Injectable depots consisting of liposomal aggregates for the sustained delivery of **peptide** and oligonucleotide drugs
AUTHOR(S): Panzner, Steffen; Lutz, Silke
CORPORATE SOURCE: ASSIGNEE: Novosom A.-G.
PATENT INFORMATION: WO 2004100928 A1 25 Nov 2004
SOURCE: (2004) PCT Int. Appl., 37 pp.
CODEN: PIXXD2.
COUNTRY: GERMANY, FEDERAL REPUBLIC OF
DOCUMENT TYPE: Patent
FILE SEGMENT: CAPLUS
OTHER SOURCE: CAPLUS 2004:1015868
LANGUAGE: German

ENTRY DATE: Entered STN: 20041207
Last Updated on STN: 20041229

TI Injectable depots consisting of liposomal aggregates for the sustained delivery of **peptide** and oligonucleotide drugs

AB The invention relates to liposomal formulations for producing an injectable depot of extended release **peptide**, protein and oligonucleotide active substances with a long-term action in a mammalian body. The liposomes include: (a) saturated synthetic phosphatidylcholins selected from the group of DMPC, DPPC and DSPC; (b) cholesterol; (c) cationic lipids selected from the group of DC-Chol, DAC-Chol, DMTAP, DPTAP, DOTAP (d) a protein or **peptide** drug. Thus recombinant insulin was encapsulated in liposomes by preparing first a dry lipid film from 60 mol% DPPC, 10 mol% DC-Chol and 30 mol% cholesterol; preparing a 50 mM insulin suspension from the lipid film and insulin solution (4 mg/mL insulin in 10 mM HEPES, 300 mM **sucrose** pH 7.5); hydratization and treatment of the suspension in ultrasound bath and in freeze-thawing cycles; extrusion and gel filtration. 80-100 % Of the insulin was encapsulated according to RP-HPLC. Similarly, leuprolide acetate was encapsulated; liposomal aggregates were injected s.c. to rats; pharmacokinetics of the system was determined

L14 ANSWER 3 OF 31 TOXCENTER COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2004:271228 TOXCENTER
COPYRIGHT: Copyright 2005 ACS
DOCUMENT NUMBER: CA14125416054K
TITLE: Injectable depots consisting of liposomal aggregates for the sustained delivery of **peptide** drugs
AUTHOR(S): Panzner, Steffen; Lutz, Silke
CORPORATE SOURCE: ASSIGNEE: Novosom A.-G.
PATENT INFORMATION: WO 2004100927 A2 25 Nov 2004
SOURCE: (2004) PCT Int. Appl., 25 pp.
CODEN: PIXXD2.
COUNTRY: GERMANY, FEDERAL REPUBLIC OF
DOCUMENT TYPE: Patent
FILE SEGMENT: CAPLUS
OTHER SOURCE: CAPLUS 2004:1015867
LANGUAGE: German
ENTRY DATE: Entered STN: 20041207
Last Updated on STN: 20041214

TI Injectable depots consisting of liposomal aggregates for the sustained delivery of **peptide** drugs

AB The invention relates to formulations of liposomes and polymers for the production of an injectable depot of active substances, having a long-term release and effect in a mammal. The anionic liposomes include: (a) saturated synthetic phosphatidylcholins selected from the group of DMPC, DPPC and DSPC; (b) cholesterol; (c) anionic lipids selected from the group of DMPG, DPPG, DSPG, DMPS and CHEMS; (d) a protein or **peptide** drug; (e) a cationic polymer. Thus recombinant insulin was encapsulated in liposomes by preparing first a dry lipid film from 50 mol% DPPC, 10 mol% DPPG and 40 mol% cholesterol; preparing a 50 mM insulin suspension from the lipid film and insulin solution (7.5 mg/mL insulin in 10 mM glycine-HCl, 300 mM **sucrose** pH 3); hydratization and treatment of the suspension in ultrasound bath and in freeze-thawing cycles; extrusion and gel filtration. 50-70 % Of the insulin was encapsulated according to ELISA. Similarly, leuprolide acetate was encapsulated; liposomal aggregates were injected s.c. to rats; pharmacokinetics of the system was determined

L14 ANSWER 4 OF 31 TOXCENTER COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2004:175885 TOXCENTER
COPYRIGHT: Copyright 2005 ACS
DOCUMENT NUMBER: CA14108128870F
TITLE: Complexes of protein crystals and ionic polymers
AUTHOR(S): Khalaf, Nazer; Govardhan, Chandrika

CORPORATE SOURCE: ASSIGNEE: Altus Biologics Inc.
PATENT INFORMATION: WO 2004060920 A1 22 Jul 2004
SOURCE: (2004) PCT Int. Appl., 80 pp.
CODEN: PIXXD2.
COUNTRY: UNITED STATES
DOCUMENT TYPE: Patent
FILE SEGMENT: CAPLUS
OTHER SOURCE: CAPLUS 2004:589569
LANGUAGE: English
ENTRY DATE: Entered STN: 20040810
Last Updated on STN: 20041221

TI Complexes of protein crystals and ionic polymers

AB The present invention relates to complexes of protein crystals and ionic polymers and compns. comprising such complexes. The invention further provides methods for producing these complexes and compns., as well as methods for treatment of an

individual having a disease requiring or ameliorated by **sustained release** of protein-based therapies. For example, human growth hormone (hGH) was purified and dissolved in water to yield a final protein concentration of 15 mg/mL.

Tris-HCl (1 M, pH 8.6) was added to a final concentration of 100 mM. To this solution, protamine sulfate was added to final concentration of 2 mg/mL. Crystals of hGH were grown by adding calcium acetate (1 M) to the solution so that a final concentration of 85 mM

calcium acetate was obtained. The solution was then incubated for 8 h at 37° to obtain needlelike crystals. The crystals obtained were found to be less than 20 μm in length with a crystallization yield of > 70%.

L14 ANSWER 5 OF 31 TOXCENTER COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2004:103629 TOXCENTER
COPYRIGHT: Copyright 2005 ACS
DOCUMENT NUMBER: CA14022363055G
TITLE: Microencapsulation and **sustained release** of biologically active **polypeptides**
AUTHOR(S): Costantino, Henry R.; Hotz, Joyce
CORPORATE SOURCE: ASSIGNEE: Alkermes Controlled Therapeutics, Inc. II
PATENT INFORMATION: WO 2004036186 A2 29 Apr 2004
SOURCE: (2004) PCT Int. Appl., 71 pp.
CODEN: PIXXD2.
COUNTRY: UNITED STATES
DOCUMENT TYPE: Patent
FILE SEGMENT: CAPLUS
OTHER SOURCE: CAPLUS 2004:355193
LANGUAGE: English
ENTRY DATE: Entered STN: 20040504
Last Updated on STN: 20041214

TI Microencapsulation and **sustained release** of biologically active **polypeptides**

AB This invention relates to compns. for the **sustained release** of biol. active polypeptides, and methods of forming and using said compns., for the **sustained** release of biol. active

polypeptides, such as glucagon, glucagon-like **peptides**, exendins, vasoactive intestinal **peptide**, Igs, antibodies, cytokines, interleukins, macrophage activating factors, interferons, erythropoietin tumor necrosis

factor, colony stimulating factors, hormones, etc. The **sustained release** compns. of this invention comprise a biocompatible polymer having dispersed therein, a biol. active **polypeptide**, a sugar and a salting-out

salt. For example, exendin-4 was encapsulated in poly(lactide-co-glycolide) using a water-oil-oil (W/O/O) emulsion system. The initial embryonic microparticles were formed in a W/O/O inner emulsion step after which they were subjected to

coacervation and hardening steps. The inner phase was prepared by dissolving the exendin-4, **sucrose** and ammonium sulfate in water or an aqueous buffer and injected into a polymer phase (PLG dissolved in methylene chloride) while

sonicating. The resultant water/oil emulsion was then mixed with silicone oil, and the mixture was added to heptene to form microparticles. The microparticles were collected, dried and filled into vials.

L14 ANSWER 6 OF 31 TOXCENTER COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2004:103618 TOXCENTER
COPYRIGHT: Copyright 2005 ACS
DOCUMENT NUMBER: CA14022363054F
TITLE: Microencapsulation and **sustained release** of biologically active **polypeptides**
AUTHOR(S): Costantino, Henry R.; Hotz, Joyce; Bobka, Edward W.
CORPORATE SOURCE: ASSIGNEE: Amylin Pharmaceuticals, Inc.
PATENT INFORMATION: WO 2004035762 A2 29 Apr 2004
SOURCE: (2004) PCT Int. Appl., 66 pp.
CODEN: PIXXD2.
COUNTRY: UNITED STATES
DOCUMENT TYPE: Patent
FILE SEGMENT: CAPLUS
OTHER SOURCE: CAPLUS 2004:355066
LANGUAGE: English
ENTRY DATE: Entered STN: 20040504
Last Updated on STN: 20041214

TI Microencapsulation and **sustained release** of biologically active **polypeptides**
AB This invention relates to compns. for the **sustained release** of biol. active polypeptides, and methods of forming and using said compns., for the **sustained** release of biol. active
polypeptides. The **sustained release** compns. of this invention comprise a biocompatible polymer having dispersed therein, a biol. active **polypeptide**, a sugar and a salting-out salt. For example,
sustained-release exendin-4 microparticles were prepared using poly(lactide-co-glycolide) (50:50), 3% exendin-4, 2% **sucrose**, and 0.3% ammonium sulfate.

L14 ANSWER 7 OF 31 TOXCENTER COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2004:103617 TOXCENTER
COPYRIGHT: Copyright 2005 ACS
DOCUMENT NUMBER: CA14022363053E
TITLE: Microencapsulation and **sustained release** of biologically active **polypeptides**
AUTHOR(S): Costantino, Henry R.; Hotz, Joyce
CORPORATE SOURCE: ASSIGNEE: Alkermes Inc.
PATENT INFORMATION: WO 2004035754 A2 29 Apr 2004
SOURCE: (2004) PCT Int. Appl., 72 pp.
CODEN: PIXXD2.
COUNTRY: UNITED STATES
DOCUMENT TYPE: Patent
FILE SEGMENT: CAPLUS
OTHER SOURCE: CAPLUS 2004:355059
LANGUAGE: English
ENTRY DATE: Entered STN: 20040504
Last Updated on STN: 20041214

TI Microencapsulation and **sustained release** of biologically active **polypeptides**
AB This invention relates to compns. for the **sustained release** of biol. active polypeptides, and methods of forming and using said compns., for the **sustained** release of biol. active
polypeptides. The **sustained release** compns. of this invention comprise a biocompatible polymer having dispersed therein, a biol. active **polypeptide**, a sugar and a salting-out salt. For example, exendin-4
was encapsulated in poly(lactide-co-glycolide) (PLG) polymer using a water-oil-oil (W/O/O) emulsion system. The initial embryonic microparticles were formed in a W/O/O inner emulsion step after which they were subjected to coacervation and

hardening steps. A water-in-oil emulsion was created using sonication. The water phase of the emulsion contained dissolved exendin-4 and excipients, e.g., sucrose and ammonium sulfate, while the PLG phase contained polymer dissolved in methylene chloride. The aqueous solution was then injected into the polymer solution while sonicating. The resultant water/oil emulsion was then mixed with silicone oil and the mixture was added to n-heptane to form microparticles. The microparticles were isolated by filtration and vacuum dried.

L14 ANSWER 8 OF 31 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-10750 BIOTECHDS
TITLE: Pharmaceutical composition useful for treating diabetes, obesity, multiple sclerosis, comprising transferrin protein exhibiting reduced glycosylation and fused to therapeutic protein or **peptide**; involving vector-mediated gene transfer and expression in yeast cell for use in disease diagnosis, prevention and therapy
AUTHOR: PRIOR C P; SADEGHI H; TURNER A
PATENT ASSIGNEE: BIOREXIS PHARM CORP
PATENT INFO: WO 2004019872 11 Mar 2004
APPLICATION INFO: WO 2003-US26778 28 Aug 2003
PRIORITY INFO: US 2003-460829 8 Apr 2003; US 2002-406977 30 Aug 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-239108 [22]
TI Pharmaceutical composition useful for treating diabetes, obesity, multiple sclerosis, comprising transferrin protein exhibiting reduced glycosylation and fused to therapeutic protein or **peptide**; involving vector-mediated gene transfer and expression in yeast cell for use in disease diagnosis, prevention and therapy
AN 2004-10750 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A pharmaceutical composition (I) formulated for oral, nasal or pulmonary delivery, comprising a transferrin (Tf) protein exhibiting reduced glycosylation and fused to a therapeutic protein or **peptide**, is new.
WIDER DISCLOSURE - (1) producing modified fusion proteins; (2) nucleic acid molecules encoding modified (II); (3) yeast cell transformed to express modified fusion proteins; and (4) beta-interferon fusion proteins
BIOTECHNOLOGY - Preferred Pharmaceutical Composition: (I) is formulated to deliver the Tf fusion protein (II) to the gastrointestinal epithelium of a patient. The Tf protein binds to the transferrin receptor present on the gastrointestinal epithelium, when orally administered to a patient. Iron is bound to the Tf protein. The Tf protein has been modified to increase its affinity or avidity for one or more of transferrin receptor, iron ions and carbonate ions.
The serum half-life of the therapeutic protein or **peptide** is increased over the serum half-life of the therapeutic protein or **peptide** in an unfused state. The therapeutic protein or **peptide** is fused to the C-terminal or N-terminal end of Tf protein. The therapeutic protein or **peptide** is inserted into a loop of the Tf protein. The Tf protein is lactotransferrin (lactoferrin) or a melanotransferrin. Tf protein comprises a mutation that reduces or prevents glycosylation. (II) is expressed in the presence of a compound that inhibits glycosylation, where the glycosylation site is chosen from amino acid residue corresponding to amino acids N413 or N611. The compound is tunicamycin. (II) comprises N-terminal to C-terminal: a therapeutic protein or **peptide**, a linker and Tf. The linker is a **peptide** that links the therapeutic protein or **peptide** to Tf. The Tf protein has a amino acid substitution, deletion or addition at position chosen from Lys 206, His 207 and its combination. The substituted amino acid is glutamine or glutamic acid. The lysine residue at amino acid position 206 is replaced with a glutamine, and the histidine residue at amino acid position 207 is replaced with a glutamic acid. The Tf protein comprises a first portion of the N domain of a Tf protein, a bridging **peptide**, and a second portion of the N domain of a Tf protein, where the first and second portions are the same. (I) comprises iron bound to the Tf or (II). The therapeutic protein or **peptide** is chosen from insulin, proinsulin, insulin analog or derivative, Glucagon Like **Peptide**-1 (GLP-1)

and a GLP-1 analog or derivative. (I) is formulated with an enteric coating. In (I), the (II) is dispersed in a carrier. The carrier is chosen from aqueous buffers, **sucrose**, lactose, starch, fatty oils, fatty acid esters, polysaccharides, monoglycerides, triglycerides, phospholipid, emulsifiers, non-ionic emulsifiers and refined colloid clays. (II) is contained in a solid form, where the solid form is tablet, chewable tablet, capsule, granulate, or a powder. The tablet or capsule is enteric coated, where the capsule is a soft gelatin capsule. The solid form is formulated for **slow release** in the gut. (II) is formulated as a liquid, aerosol or syrup. The formulated (I) comprise 1 pg/kg-100 mg/kg, preferably 100 ng/kg-100 micrograms/kg, and most preferably 100 micrograms/kg-100 mg/kg body weight of (II). (I) comprises 1 micrograms-1 g, preferably 10 micrograms-100 mg, and most preferably 10 mg-50 mg of (II). (I) for oral delivery comprises Tf protein exhibiting reduced glycosylation and fused to a insulin or GLP-1 protein or its **peptide**. The insulin protein or **peptide** is human insulin, proinsulin or mature human insulin. The human insulin comprises Arg-Ser-Leu-Glu-Lys-Arg-Val-Pro-Asp. The Tf comprises iron or carbonate ions. (I) containing insulin protein is formulated to comprise 5-500 units, preferably 10-100 units of insulin per kg of patient weight. (I) containing insulin or GLP-1 protein, or its **peptide**, comprises an effective amount of insulin or GLP-1 to induce a decrease in blood glucose, and to induce a hypoglycemic effect in a patient, where the effective amount decreases blood glucose levels by 5%-80%. The GLP-1 protein or **peptide** is human GLP-1 amino acids 7-35, 7-36 or 7-37, preferably 7-37. The insulin or GLP-1 protein, or its **peptide** is fused to the N-terminal end of Tf. The insulin or GLP-1 protein, or its **peptide** is separated from the N-terminal end of Tf by a linker **peptide**. (I) containing insulin or GLP-1 protein, or its **peptide** is formulated to neutralize or protect the fusion protein from gastric acid and/or enzymes, and further comprises a transcytosis enhancer. (I) comprise an effective amount of (II) to increase the serum insulin or GLP-1 activity level in a patient. GLP-1 protein or **peptide** has modified N-terminal end to prevent cleavage, where the modification is a amino acid substitution. The formulation comprises iron and carbonate bound to transferrin. The mutation is within the N-X-S/T glycosylation site, where Ser or Thr is mutated. X is mutated to Pro.

The GLP-1 analog is exendin. The GLP-1 protein or **peptide** comprises the second residue from the N-terminus is substituted with another amino acid. The Tf protein comprises a single N domain.

ACTIVITY - Virucide; Immunosuppressive; Cytostatic; Anorectic; Antiinflammatory; Antimicrobial; Antianemic; Antidiabetic; Neuroprotective; Antisickling; Hemostatic; Anti-HIV; Nootropic; Nephrotropic; Coccidiostatic; Protozoacide. No biological data given.

MECHANISM OF ACTION - Non-given.

USE - (I) comprising (II) is effective to treat a human disease, preferably chronic human disease. The chronic disease is chosen from viral disease, cancer, metabolic disease, obesity, autoimmune disease, inflammatory disease, allergy, graft-vs.-host disease, systemic microbial infection, anemia, cardiovascular disease, neurodegenerative disease, disorder of hematopoietic cells, diseases of the endocrine system or reproductive systems, gastrointestinal disease, diabetes and multiple sclerosis. (I) is formulated by mixing with food or a beverage, as a feed supplement for veterinary use. (I) is useful for administering a therapeutic protein or **peptide** to a patient in need, which involves orally administering (I). (I) is useful for enhancing the absorption of a therapeutic protein or **peptide** into the bloodstream from the digestive tract of a patient, which involves administering (II), where the therapeutic protein or **peptide** is absorbed into the bloodstream at an increased rate compared to absorption of the therapeutic protein or **peptide** in an unfused state. The enhancing and administering method further involves administering a transcytosis enhancer. (I) is useful for treating diabetes in a patient, which involves orally administering (II) that comprises Tf protein fused to insulin or GLP-1 protein, or its **peptide**. (I) is useful for enhancing the absorption

of an insulin or GLP-1 protein, or its **peptide** into the bloodstream from the digestive tract of a diabetic patient, which involves administering (II) that comprises Tf protein fused to insulin or GLP-1 protein, or its **peptide**

. (I) is also useful for altering the blood glucose levels in a patient, which involves administering (II) that comprises Tf protein fused to insulin or GLP-1 protein or **peptide**. The patient is human, preferably juvenile or adult.

The patient is a diabetic patient and has juvenile or adult onset diabetes, preferably Type II diabetes. The patient is diabetic or obese (claimed). (I) is useful for treating, preventing and/or diagnosing tissue specific inflammatory

disorders such as alveolitis, angiocholecystitis, appendicitis, etc., anemia such as hypochromic anemia, microcytic anemia, iron deficiency anemia, etc., hemoglobin abnormalities such as sickle cell anemia, hemoglobin E disease,

thromobocytopenia, hyperproliferative disorders, cancer such as Acute lymphoblastic leukemia, Acute myeloid leukemia, etc., AIDS, neurodegenerative disorders such as Alzheimers disease, Parkinsons disease, etc., sclerotic or lecrotic disorders

of the kidney, cardiovascular disorders such as pulmonary atresia, congenital heart defects, respiratory disorders such as Goodpastures syndrome, Pneumonia, etc., cerebrovascular diseases, parasitic infections such as Leishmaniasis,

Coccidiosis, etc.

ADMINISTRATION - (I) is administered orally, nasally, or pulmonarally (claimed). (I) is administered intravenously, intramuscularly, intraperitoneally, intradermally, subcutaneously, rectally or transdermally. The dosage range for oral

administration is 50-100 mg/day, preferably 200 mg/day.

ADVANTAGE - (I) enables disease prevention, disease stabilization, the lessening or alleviation of disease symptoms or a modulation, alleviation or cure of the underlying defect to produce an effect beneficial to the treated subject.

EXAMPLE - The cDNA was generated from a cDNA pool by reverse transcriptase-polymerase chain reaction (RT-PCR) by using the primers having the sequences 5'-tttgtgaaccaacacctgtgcggc-3' and 3'-gacgagggagatggtcgacctcttgatgacgttg-5'. The

N-terminal insert was produced using the 5' mutagenic primer having sequences 5'-gcttactctaggtctctagataagaggtttgtgaaccaacacctgtgcg-3', and the first PCR product as template. The primer inserted the last 5 amino acids of the leader sequence and

the XbaI site. The PCR product was digested with XbaI/PvuII. A linker was then made of two overlapping oligos with a PvuII 5' end and 3' overhang which ligate to the KpnI overhang or KpnI digested pREX0052. By annealing and ligating this

linker to the digested PCR fragment and ligating the resulting product into XbaI/KpnI digested pREX0052, the plasmid pREX0052 N-insulin was generated. The C-terminal insert was generated using 5' and 3' mutagenic primers, and the first PCR

product as template. The obtained PCR product was then digested with SalI/HindIII and ligated into SalI/HindIII digested pREX0052. Thus the plasmid pREX0052 C-insulin was obtained. DNA sequence for both N- and C-terminal inserts had been

checked and confirmed, the plasmids pREX0052 N-insulin and pREX0052 C-insulin were digested with NotI and the expression cassettes were recovered. These were then ligated into NotI digested pSAC35 to obtain pSAC35 N-insulin and pSAC35

C-insulin. These plasmids were then electroporated into the host Saccharomyces yeast strains and transformants selected for leucine prototrohy on minimal media plates. Expression was determined by growth in liquid minimal media and the

superanatant was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), western blot, ELISA and BIAcore. The results showed that the fusion constructs resulted in production of proinsulin attached to transferrin. The

obtained proinsulin attached to transferrin fusion protein was adsorbed by the intestinal mucosa and exhibited insulin activity, when injected intraperitoneally into the diabetic rats.(356 pages)

L14 ANSWER 9 OF 31 TOXCENTER COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:302917 TOXCENTER

COPYRIGHT: Copyright 2005 ACS
DOCUMENT NUMBER: CA14002019881U
TITLE: Hazard-free microencapsulation for structurally delicate agents, an application of stable aqueous-aqueous emulsion
AUTHOR(S): Jin, Tuo; Zhu, Hua; Zhu, Jiahao
PATENT INFORMATION: WO 2003101600 A2 11 Dec 2003
SOURCE: (2003) PCT Int. Appl., 49 pp.
CODEN: PIXXD2.
COUNTRY: CHINA
DOCUMENT TYPE: Patent
FILE SEGMENT: CAPLUS
OTHER SOURCE: CAPLUS 2003:971951
LANGUAGE: English
ENTRY DATE: Entered STN: 20031223
Last Updated on STN: 20041207

TI Hazard-free microencapsulation for structurally delicate agents, an application of stable aqueous-aqueous emulsion

AB This invention provides method for **sustained release** delivery of structurally delicate agents such as proteins and **peptides**. Using a unique emulsion system (stable polymer aqueous-aqueous emulsion), proteins and **peptides** can be microencapsulated in polysaccharide glassy particles under a condition free of any chemical or phys. hazard such as organic solvents, strong interfacial tension, strong shears, elevated temperature, large amount of surfactants, and crosslinking agents. Proteins loaded in these glassy particles showed strong resistance to organic solvents, prolonged activity in hydrated state, and an excellent **sustained release** profile with minimal burst and incomplete release when being further loaded in degradable polymer microspheres. This invention provides a simple yet effective approach to address all the tech. challenges raised in **sustained release** delivery of proteins.

L14 ANSWER 10 OF 31 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-27882 BIOTECHDS
TITLE: New secreted transmembrane **polypeptides** and nucleic acids encoding the **polypeptides**, useful in gene therapy, in identifying chromosomes, as chromosome markers, in generating probes and in tissue typing; recombinant protein production and antagonist and agonist for use in disease therapy and gene therapy
AUTHOR: ASHKENAZI A J; BAKER K P; BOTSTEIN D; DESNOYERS L; EATON D L; FERRARA N; FONG S; GERBER H; GERRITSEN M E; GODDARD A; GODOWSKI P J; GRIMALDI J C; GURNEY A L; KLJAVIN I J; NAPIER M A; PAN J; PAONI N F; ROY M A; STEWART T A; TUMAS D; WATANABE C K; WILLIAMS P M; WOOD W I; ZHANG Z
PATENT ASSIGNEE: GENENTECH INC
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TI New secreted transmembrane **polypeptides** and nucleic acids encoding the polypeptides, useful in gene therapy, in identifying chromosomes, as chromosome markers, in generating probes and in tissue typing; recombinant protein production and antagonist and agonist for use in disease therapy and gene therapy

AN 2003-27882 BIOTECHDS

AB DERWENT ABSTRACT:
NOVELTY - An isolated nucleic acid (I) encoding a PRO **polypeptide**, is new.
DETAILED DESCRIPTION - An isolated nucleic acid (I) encoding a PRO polypeptide, is new. (I) has at least 80 % sequence identity to: (a) a sequence encoding a **polypeptide** having one of 147 75-850 residue amino acid sequences (P1), given in the specification; (b) a sequence selected from 147 750-3500 nucleotide sequences (S1), given in the specification; (c) the full length coding sequence of S1 or of the DNA deposited under ATCC (accession numbers given

in the specification); or (d) a nucleotide sequence encoding an extracellular domain of P1 with or without its associated signal **peptide**. INDEPENDENT CLAIMS are also included for the following: (1) a vector comprising (I); (2) a host cell comprising the vector of (1); (3) producing a PRO **polypeptide** by culturing the host cell of (2) for the expression of the PRO **polypeptide**, and recovering the PRO **polypeptide** from the cell culture; (4) an isolated PRO **polypeptide** having at least 80 % sequence identity to: (a) an amino acid sequence selected from P1; (b) an amino acid sequence encoded by the full length coding sequence of the DNA deposited under ATCC; or (c) an amino acid of an extracellular domain of a **polypeptide** of (a) having or lacking its associated signal **peptide**; (5) a chimeric molecule comprising a PRO **polypeptide** of (4) fused to a heterologous amino acid sequence; (6) an antibody, which specifically binds to a PRO **polypeptide**; (7) detecting a PRO943, PRO183, PRO184, PRO185, PRO331, PRO1113, PRO363, PRO5723, PRO1387, PRO1114, PRO3301, PRO9940, PRO1181, PRO7170, PRO361, or PRO846 **polypeptide** in a sample suspected of containing the **polypeptide**; (8) linking a bioactive molecule to a cell expressing a PRO943, PRO183, PRO184, PRO185, PRO331, PRO113, PRO363, PRO5723, PRO1387, PRO1114, PRO3301, PRO9940, PRO1181, PRO7170, PRO361, or PRO846 **polypeptide**; and (9) modulating at least one biological activity of a cell expressing a PRO943, PRO183, PRO184, PRO185, PRO331, PRO113, PRO363, PRO5723, PRO1387, PRO1114, PRO3301, PRO9940, PRO1181, PRO7170, PRO361, or PRO846 **polypeptide**.

WIDER DISCLOSURE - (1) agonists and antagonists of the **polypeptides**; and (2) identifying agonists and antagonists.

BIOTECHNOLOGY - Preferred Host Cell: The host cell is a Chinese hamster ovary (CHO) cell, an Escherichia coli cell or a yeast cell. Preferred Chimeric Molecule: The heterologous amino acid sequence is an epitope tag sequence or an Fc region of an immunoglobulin. Preferred Antibody: The antibody is a monoclonal, humanized or an antibody fragment. Preferred Method: Detecting a PRO943 polypeptide in a sample containing the **polypeptide** comprises contacting the sample with a PRO183, PRO184 or PRO185 **polypeptide**, and determining the formation of a PRO943/PRO183, PRO184 or PRO185 **polypeptide** conjugate in the sample indicating the presence of the PRO943 **polypeptide**. The sample comprises cells expressing PRO943 **polypeptide**. PRO183, PRO184 or PRO185 **polypeptide** is labeled with a detectable label or is attached to a solid support. In detecting a PRO183, PRO184 or PRO185 **polypeptide** in a sample, the sample is contacted with PRO943, where formation of a PRO943/PRO183, PRO184 or PRO185 **polypeptide** conjugate indicates the presence of PRO183, PRO184 or PRO185 **polypeptide** in the sample. The sample comprises cells expressing PRO183, PRO184 or PRO185 **polypeptide**. PRO943 is labeled with a detectable label or is attached to a solid support. Detecting a PRO331 or PRO1133 polypeptide in a sample comprises contacting the sample with PRO1133 or PRO331, respectively, where formation of a PRO331/PRO1133 polypeptide conjugate indicates the presence of the **polypeptide** in the sample. Detecting a PRO363 or PRO5723 **polypeptide** in a sample containing the **polypeptide** comprises contacting the sample with a PRO1387 **polypeptide**, and determining the formation of a PRO363 or PRO5723/PRO1387 **polypeptide** conjugate in the sample indicating the presence of the PRO331 or PRO1133 **polypeptide**. Detecting a PRO1387 **polypeptide** in a sample containing the **polypeptide** comprises contacting the sample with a PRO363 or PRO5723 **polypeptide**, and determining the formation of a PRO363 or PRO5723/PRO1387 **polypeptide** conjugate in the sample, which indicates the presence of the PRO1387 **polypeptide**. Detecting a PRO1114 **polypeptide** in a sample containing the **polypeptide** comprises contacting the sample with a PRO3301 or PRO9940 **polypeptide**, and determining the formation of a PRO1114/PRO3301 or PRO9940 **polypeptide** conjugate in the sample, which indicates the presence of the PRO1114 **polypeptide**. Detecting a PRO3301 or PRO9940 **polypeptide** in a sample containing the polypeptide comprises contacting the sample with a PRO1114 **polypeptide**, and determining the formation of a PRO1114/PRO3301 or PRO9940 **polypeptide** conjugate in the sample which indicates the presence of the PRO3301 or PRO9940 **polypeptide**. Detecting a PRO1181 **polypeptide** in a sample containing the **polypeptide** comprises contacting the

sample with a PRO7170, PRO361 or PRO846 **polypeptide**, and determining the formation of a PRO1181/ PRO7170, PRO361 or PRO846 **polypeptide** conjugate in the sample, which indicates the presence of the PRO1181 **polypeptide**

. Detecting a PRO7170, PRO361 or PRO846 **polypeptide** in a sample containing the **polypeptide** comprises contacting the sample with a PRO1181 **polypeptide**, and determining the formation of a PRO1181/ PRO7170, PRO361 or

PRO846 **polypeptide** conjugate in the sample which indicates the presence of the PRO7170, PRO361 or PRO846 **polypeptide**. The sample comprises cells suspected of expressing the **polypeptide** to be detected, and is

contacted with a **polypeptide** labeled with a detectable label or which is attached to a solid support. Linking a bioactive molecule to a cell expressing a PRO943 **polypeptide** comprises contacting the cell with a PRO183,

PRO184 or PRO185 **polypeptide** that is bound to the bioactive molecule, and allowing the **polypeptides** to bind to one another. Linking a bioactive molecule to a cell expressing a PRO183, PRO184 or PRO185 **polypeptide**

comprises contacting the cell with a PRO943 **polypeptide** that is bound to the bioactive molecule, and allowing the **polypeptides** to bind to one another. Linking a bioactive molecule to a cell expressing a PRO3301 or PRO1133

polypeptide comprises contacting the cell with a PRO1133 or PRO3301 polypeptide, respectively, bound to the bioactive molecule, and allowing the polypeptides to bind to one another. Linking a bioactive molecule to a

cell expressing a PRO1387 **polypeptide** comprises contacting the cell with a PRO363 or PRO5723 **polypeptide** bound to the bioactive molecule, and allowing the polypeptides to bind to one another. Linking a bioactive

molecule to a cell expressing a PRO363 or PRO5723 **polypeptide** comprises contacting the cell with a PRO1387 **polypeptide** bound to the bioactive molecule, and allowing the **polypeptides** to bind to one another. Linking

a bioactive molecule to a cell expressing a PRO1144 **polypeptide** comprises contacting the cell with a PRO3301 or PRO9940 **polypeptide** bound to the bioactive molecule, and allowing the **polypeptides** to bind to one

another. Linking a bioactive molecule to a cell expressing a PRO3301 or PRO9940 **polypeptide** comprises contacting the cell with a PRO1144 **polypeptide** bound to the bioactive molecule, and allowing the **polypeptides**

to bind to one another. Linking a bioactive molecule to a cell expressing a PRO1181 **polypeptide** comprises contacting the cell with a PRO7170, PRO361 or PRO846 polypeptide, bound to the bioactive molecule, and allowing the

polypeptides to bind to one another. Linking a bioactive molecule to a cell expressing a PRO7170, PRO361 or PRO846 **polypeptide** comprises contacting the cell with a PRO1181 **polypeptide** bound to the bioactive

molecule, and allowing the **polypeptides** to bind to one another. The bioactive molecule is a toxin, a radiolabel or an antibody. The bioactive molecule may cause the death of the cell. Modulating at least one biological activity of a

cell expressing PRO943 **polypeptide** comprises contacting the cell with a PRO183, PRO184 or PRO185 **polypeptide**, or an anti-PRO943 antibody, where the polypeptide or the antibody binds to PRO943 **polypeptide**

to modulate at least one biological activity of the cell, where the cell is killed. Modulating at least one biological activity of a cell expressing a PRO183, PRO184 or PRO185 **polypeptide** comprises contacting the cell with a PRO943

polypeptide, or an anti-PRO183, anti-PRO184 or anti-PRO185 antibody, where the **polypeptide** or antibody binds to PRO183, PRO184 or PRO185 **polypeptide** to modulate at least one biological activity of the cell, where

the cell is killed. Modulating at least one biological activity of a cell expressing a PRO1133 or PRO331 **polypeptide** comprises contacting the cell with a PRO331 or PRO1133 **polypeptide**, or an anti-PRO331 or anti-PRO1133

antibody, where the **polypeptide** or antibody binds to PRO1133 or PRO331 polypeptide to modulate at least one biological activity of the cell, where the cell is killed. Modulating at least one biological activity of a cell

expressing PRO1387 **polypeptide** comprises contacting the cell with a PRO363 or PRO5723 **polypeptide**, or an anti-PRO1387 antibody, where the **polypeptide** or the antibody binds to PRO1387 **polypeptide** to

modulate at least one biological activity of the cell, where the cell is killed. Modulating at least one biological activity of a cell expressing PRO363 or PRO5723 **polypeptide** comprises contacting the cell with a PRO1387

polypeptide, or an anti-PRO363 or anti-PRO5723 antibody, where the **polypeptide** or the antibody binds to PRO363 or PRO5723 **polypeptide** to modulate at least one biological activity of the cell, where the cell is killed. Modulating at least one biological activity of a cell expressing PRO1114 **polypeptide** comprises contacting the cell with a PRO3301 or PRO9940 polypeptide, or an anti-PRO1114 antibody, where the **polypeptide** or the antibody binds to PRO1114 **polypeptide** to modulate at least one biological activity of the cell, where the cell is killed. Modulating at least one biological activity of a cell expressing PRO3301 or PRO9940 **polypeptide** comprises contacting the cell with a PRO1114 **polypeptide**, or an anti-PRO3301 or anti-PRO9940 antibody, where the **polypeptide** or the antibody binds to PRO3301 or PRO9940 **polypeptide** to modulate at least one biological activity of the cell, where the cell is killed. Modulating at least one biological activity of a cell expressing PRO1181 **polypeptide** comprises contacting the cell with a PRO7170, PRO361 or PRO846 **polypeptide**, or an anti-PRO1181 antibody, where the **polypeptide** or the antibody binds to PRO1181 **polypeptide** to modulate at least one biological activity of the cell, where the cell is killed. Modulating at least one biological activity of a cell expressing PRO7170, PRO361 or PRO846 **polypeptide** comprises contacting the cell with a PRO1181 **polypeptide**, or an anti-PRO7170, anti-PRO361 or anti-PRO846 antibody, where the **polypeptide** or the antibody binds to PRO7170, PRO361 or PRO846 **polypeptide** to modulate at least one biological activity of the cell, where the cell is killed.

ACTIVITY - None given.

MECHANISM OF ACTION - Gene therapy. No biological data is given.

USE - The nucleotide sequences are useful as probes, in chromosome and gene mapping, in generating antisense RNA and DNA, in preparing PRO **polypeptides** by recombinant techniques, and in gene therapy (e.g. for replacement of defective gene). The PRO **polypeptides** are useful as molecular weight markers for protein electrophoresis purposes, for chromosome identification, as chromosome markers, as therapeutic agents, for stimulating the release of tumor necrosis factor (TNF)-alpha from human blood, for stimulating the proliferation or differentiation of chondrocytes, and detecting the presence of tumor. The PRO **polypeptides** and nucleic acids may also be used diagnostically for tissue typing.

ADMINISTRATION - Dosage is 10 ng-100 mg/kg, preferably 1 micro-g-10 mg/kg/day. Administration can be through injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial or intralesional routes, topical, or by **sustained release** systems.

EXAMPLE - Yeast transformation was performed with limiting amounts of transforming DNA to reduce the number of multiple transformed yeast cells. Instead of plasmid isolation form the yeast followed by transformation of Escherichia coli, PCR was performed on single yeast colonies using bipartite primers to amplify the insert and a small portion of the invertase gene and to add on universal sequencing primer sites. An invertase library was transformed into yeast and positives were selected on **sucrose** plates. Positive clones were re-tested and PCR products were sequenced. The sequence of one clone, PRO281, was determined to contain a signal **peptide** coding sequence. Oligonucleotide primers and probes were designed using the nucleotide sequence of PRO281. A full-length plasmid library of cDNAs from human umbilical vein endothelium tissue was tittered and about 100000 colony forming units (cfu) were plated in 192 pools of 500 cfu/pool into 96-2311 round bottom plates, and were incubated overnight with shaking. PCR was performed on individual cultures and agarose gel electrophoresis was performed where positive wells were identified by visualization of a band of expected size. Individual positive clones were obtained by colony lift followed by hybridization with 32-labeled oligonucleotide. Clones were characterized by PCR, restriction digest and southern blot. A full-length clone was identified and contained a single open reading frame with an apparent translational initiation site at nucleotide positions 80-82, and a stop signal at nucleotide positions 1115-1117. The entire nucleotide sequence consisted of 1943 base pairs, and the predicted

polypeptide precursor is 345 amino acids long.(648 pages)

L14 ANSWER 11 OF 31 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-20475 BIOTECHDS
TITLE: Novel isolated PRO **polypeptides** e.g., PRO826, PRO1068, PRO1184, PRO1346 and PRO1375, which stimulate proliferation of stimulated T-lymphocytes and are thus therapeutically useful for enhancing immune response;
involving vector-mediated gene transfer and expression in Chinese hamster ovary, Escherichia coli or yeast cell for use in gene therapy
AUTHOR: ASHKENAZI A J; BAKER K P; BOTSTEIN D; DESNOYERS L; EATON D L; FERRARA N; FONG S; GERBER H; GERRITSEN M E; GODDARD A; GODOWSKI P J; GRIMALDI J C; GURNEY A L; KLJAVIN I J; NAPIER M A; PAN J; PAONI N F; ROY M A; STEWART T A; TUMAS D;
WATANABE C K; WILLIAMS P M; WOOD W I; ZHANG Z
PATENT ASSIGNEE: GENENTECH INC
PATENT INFO: US 2003059832 27 Mar 2003
APPLICATION INFO: US 2001-997349 15 Nov 2001
PRIORITY INFO: WO 2001-21735 9 Jul 2001; WO 1997-20069 5 Nov 1997
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OTHER SOURCE: WPI: 2003-540678 [51]
TI Novel isolated PRO **polypeptides** e.g., PRO826, PRO1068, PRO1184, PRO1346 and PRO1375, which stimulate proliferation of stimulated T-lymphocytes and are thus therapeutically useful for enhancing immune response;
involving vector-mediated gene transfer and expression in Chinese hamster ovary, Escherichia coli or yeast cell for use in gene therapy
AN 2003-20475 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An isolated PRO **polypeptide** (I) having at least 80% sequence identity to: (i) an amino acid sequence chosen from any one of 147 fully defined polypeptide sequences (PS) as given in the specification; or (ii) PS lacking its associated signal **peptide** or an isolated extracellular domain of PRO **polypeptide** with or without its associated signal **peptide**, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid (II) having at least 80% identity to: (a) a nucleotide sequence encoding a sequence of PS; (b) a nucleic acid which comprises any one of 147 fully defined PRO polynucleotide sequences (NS) as given in the specification; (c) full-length coding sequence of NS; or (d) nucleotide sequence encoding: (i) PS lacking its associated signal **peptide**; or (ii) an extracellular domain of PS with or without its associated signal **peptides**; (2) isolated nucleic acid which comprises the full-length coding sequence of DNA deposited under any one of 141 ATCC Accession number, as given in the specification; (3) a vector (III) comprising (II) having at least 80% sequence identity to nucleotide sequence encoding PS; (4) a host cell (IV) comprising (III); (5) preparation of (I); (6) an isolated PRO **polypeptide** having at least 80% sequence identity to the amino acid sequence encoded by a nucleic acid molecule deposited under any of 141 ATCC accession numbers as described above; (7) a chimeric molecule (V) comprising (I) which has 80% sequence identity to PS, fused to a heterologous amino acid sequence; (8) an antibody (VI) which specifically binds to (I) which has 80% sequence identity to PS; (9) an isolated extracellular domain (VIII) of the PRO **polypeptide**; (10) an isolated PRO **polypeptide** (IX) lacking its associated signal **peptide**; and (11) an isolated polypeptide having at least 80% amino acid sequence identity (VIII) or (IX).
WIDER DISCLOSURE - The following are disclosed: (a) nucleic acids complementary to above mentioned nucleic acids; (b) fragments of PRO **polypeptide** coding sequence; (c) a composition comprising PRO **polypeptide**, its agonists or antagonists, anti-PRO **polypeptide** useful in treatment of conditions responsive to PRO **polypeptide**, its agonists or antagonists or anti-PRO antibodies; (d) agonists and antagonists of PRO **polypeptides**; (e) variants of (I); and (f) covalent modifications of (I).
BIOTECHNOLOGY - Preparation: (I) is prepared by standard recombinant techniques (claimed). Preferred Polynucleotide: (II) comprises a full-length coding sequence of NS. Preferred Vector: (III) comprises (II) operably linked to the control

sequences recognized by a host cell transformed with the vector. Preferred Host Cell: (IV) is a Chinese hamster ovary cell, yeast cell or an Escherichia coli cell. Preferred Molecule: (V) comprises (I) fused to an epitope tag sequence or to an

immunoglobulin Fc region.

ACTIVITY - Vulnerary; Cytostatic; Antiarthritic; Ophthalmological; Nephrotropic.

MECHANISM OF ACTION - PRO antagonist; Stimulates hypertrophy of neonatal heart; Stimulates adrenal cortical capillary endothelial growth; Inhibits vascular endothelial growth factor (VEGF) stimulated proliferation of endothelial cells;

Stimulates proliferation of stimulated T-lymphocytes; Enhances survival of rod photoreceptor cells or neuronal cells; Induces proliferation of mammalian kidney mesangial cells; Induces proliferation and/or redifferentiation of chondrocytes in

culture. The ability of the PRO **polypeptides** PRO1310, PRO844, PRO1312, PRO1192 and PRO1387 to induce the proliferation and/or redifferentiation of chondrocyte in culture was tested in a chondrocyte proliferation assay. Porcine

chondrocytes were isolated by overnight collagenase digestion of articular cartilage of the metacarpophalngeal joint of 4-6 month old female pigs. The isolated cells were then seeded at 25000 cells/cm2 in Ham F-12. On day 12, the cells were

seeded in 96-well plates at 5000 cells/well in 100microl of the media without serum and 100 microl of either serum-free medium, staurosporin, or the test PRO polypeptide. After 5 days, at 37degreesC, 2 microl of Alamar blue was added

to each well and the plates were incubated for an additional 3 hours at 37degreesC. The fluorescence was then measured in each well. The PRO **polypeptide** -treated sample provided a positive result, i.e., a fluorescence more likely that

of the positive control than the negative control.

USE - (I) is useful for detecting the PRO **polypeptides** in sample e.g., PRO943 **polypeptide** is detected in a sample suspected of containing a PRO943 polypeptide, by contacting the sample with a PRO183, PRO184

or PRO185 **polypeptide** and determining the formation of PRO943/PRO183, PRO184, or PRO185 **polypeptide** conjugate; and the presence of PRO183, PRO184 or PRO185 polypeptide in a sample is detected by contacting PRO943

polypeptide and determining the formation of the conjugate as described above. Similarly, the **polypeptides** PRO331; PRO363 or PRO5723; PRO1114; PRO1181 are detected by contacting sample with PRO1133; PRO1387; PRO3301 or

PRO9940; PRO7170, PRO361 or PRO846, **polypeptides**, respectively and determining formation of conjugate. And for detecting PRO1133; PRO1387; PRO3301 or PRO9940; PRO7170, PRO361 or PRO846, **polypeptides**, the sample is contacted

with PRO331; PRO363 or PRO5723; PRO1114; PRO1181 **polypeptides**, respectively, and formation of conjugate is determined. The sample comprises cells suspected of expressing the PRO **polypeptide**. PRO **polypeptides** which

are contacted with the sample are labeled with a detectable label or a solid support. The PRO **polypeptides** are also useful for linking a bioactive molecule to a cell expressing a PRO **polypeptide**, e.g. PRO183, PRO184 or

PRO185; PRO1133; PRO363 or PRO5723; PRO3301 or PRO9940; PRO7170, PRO361 or PRO846, **polypeptides** are useful for linking a bioactive molecule to a cell expressing PRO943; PRO331; PRO1387; PRO1114; PRO1181 **polypeptides**,

respectively, and the **polypeptides** PRO943; PRO331; PRO1387; PRO1114; PRO1181 are useful for linking a bioactive molecule to a cell expressing PRO183, PRO184 or PRO185; PRO1133; PRO363 or PRO5723; PRO3301 or PRO9940; PRO7170, PRO361

or PRO846, **polypeptides**, respectively. The bioactive molecule is a toxin, radiolabel or antibody, which causes the death of the cell. The PRO **polypeptides** are useful for modulating a biological activity of a cell expressing

the PRO **polypeptides**, e.g. the biological activity of a cell expressing PRO183, PRO184 or PRO185 is modulated by contacting the cell with PRO943 **polypeptide** or anti-PRO183, anti-PRO184 or anti-PRO185 antibody, such that the

cell is killed. Similarly, other PRO **polypeptides** are useful for modulating the biological activity of the cell expressing the counterpart **polypeptides** as described above (all claimed). The PRO **polypeptides** are

also useful for treating cardiac insufficiency disorders, wound healing, inhibiting tumor growth, enhancing immune response, treating retinal disorders or injuries, e.g. sight loss in mammals, treating retinitis pigmentosum, age-related

macular degeneration, kidney disorders associated with decreased mesangial cell function such as Berger disease or other nephropathies associated with dermatitis, herpetiformis or Crohn's disease.

ADMINISTRATION - Administered by injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial or intralesional route, topical route or by **sustained release** systems.

The PRO **polypeptide** is administered at a dose of 1 ng/kg-100 mg/kg of mammal body weight or more/day, preferably 1 microg/kg/day-10 mg/kg/day.

EXAMPLE - Isolation of cDNA clones encoding human PRO281 was carried out as follows. In order to obtain a cDNA clone encoding PRO281, methods described in Klein et al., Proc. Natl. Acad. Sci. USA 93:7108-7113 (1996) were employed with the following modifications. Yeast transformation was performed with limiting amounts of transforming DNA in order to reduce the number of multiple transformed yeast cells. Instead of plasmid isolation from the yeast followed by transformation of

Escherichia coli. Polymerase chain reaction (PCR) analysis was performed on single yeast colonies. PCR primers employed were bipartite in order to amplify the insert and a small portion of the invertase gene (allowing to determine that the insert was in frame with invertase) and to add on universal sequencing primer sites. An invertase library was transformed into yeast and positives were selected on **sucrose** plates. Positive clones were re-tested and PCR products were sequenced. The sequence of one clone, PRO281, was determined to contain a signal **peptide** coding sequence. Oligonucleotide primers and probes were designed using the nucleotide sequence of PRO281. A full length plasmid library of cDNAs from human umbilical vein endothelium tissue was titered and 100000 cfu (colony forming units) were plated in 192 pools of 500 cfu/pool into 96-well round bottom plates. The plates were sealed and pools were grown overnight at 37degreesC with shaking. PCR was performed on the individual cultures using primers. Agarose gel electrophoresis was performed and positive wells were identified. Individual positive clones were obtained by colony lift followed by hybridization with

32P-labeled oligonucleotide. These clones were characterized by PCR, restriction digest, and southern blot analyses. A full length clone was identified that contained a single open reading frame with an apparent translational initiation site

at nucleotide positions 80-82 and a stop signal at nucleotide positions 1115-1117. The predicted **polypeptide** precursor is 345 amino acids (S1) long, has a calculated molecular weight of 37205 Da and an estimated pI of 10.15. Analysis of the full-length PRO281 sequence of (S1) showed the presence of the following: a signal **peptide** from amino acid 1-14, multiple transmembrane domains from amino acids 83-105, 126-146, 158-177, 197-216, 218-238, 245-265, 271-290, and an amino acid sequence block having homology to G-protein coupled receptor proteins from amino acid 115-155. Clone UNQ244 (DNA16422-1209) was deposited with ATCC on Jun 2, 1998 and is assigned ATCC deposit no.209929. An analysis of the Dayhoff

database (version 35,45 SwissProt 25), using a WU-BLAST-2 sequence alignment analysis of the full-length sequence of PRO281, evidenced significant homology between the PRO281 amino acid sequence and the following Dayhoff sequences: H64634, AF0330951, B64815, YBHLE-COLI, EMEQUTR1, AF0647633, S53708, A69253, AF03541312 and S63281. (654 pages)

L14 ANSWER 12 OF 31 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-20474 BIOTECHDS
TITLE: Novel secreted and transmembrane **polypeptide** for modulating biological activity of cell expressing the **polypeptide**, identifying agonists or antagonists of **polypeptide**, and as molecular weight markers;
involving vector-mediated gene transfer and expression in Chinese hamster ovary, Escherichia coli or yeast cell for use in gene therapy
AUTHOR: ASHKENAZI A J; BAKER K P; BOTSTEIN D; DESNOYERS L; EATON D L; FERRARA N; FONG S; GERBER H; GERRITSEN M E; GODDARD A; GODOWSKI P J; GRIMALDI J C; GURNEY A L; KLJAVIN I J; NAPIER M A; PAN J; PAONI N F; ROY M A; STEWART T A; TUMAS D;

WATANABE C K; WILLIAMS P M; WOOD W I; ZHANG Z
PATENT ASSIGNEE: GENENTECH INC
PATENT INFO: US 2003059831 27 Mar 2003
APPLICATION INFO: US 2001-989729 19 Nov 2001
PRIORITY INFO: WO 2001-21735 9 Jul 2001; WO 1997-20069 5 Nov 1997
DOCUMENT TYPE: Patent
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OTHER SOURCE: WPI: 2003-540677 [51]

TI Novel secreted and transmembrane **polypeptide** for modulating biological activity of cell expressing the **polypeptide**, identifying agonists or antagonists of polypeptide, and as molecular weight markers;
involving vector-mediated gene transfer and expression in Chinese hamster ovary, Escherichia coli or yeast cell for use in gene therapy

AN 2003-20474 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - An isolated, secreted and transmembrane **polypeptide**, termed PRO polypeptide (I) having at least 80% sequence identity to a sequence (S1) selected from any one of the 147 sequences fully defined in the specification or to a sequence (S2) encoded by a nucleic acid molecule deposited under any one of the ATCC accession numbers given in the specification, is new.

DETAILED DESCRIPTION - (I) comprises: (a) an isolated **polypeptide** having at least 80% sequence identity to S1 or S2; (b) an isolated extracellular domain of the PRO **polypeptide**; (c) an isolated PRO **polypeptide** lacking its associated signal **peptide**; (d) an isolated **polypeptide** having at least 80% sequence identity to (b) or (c); and/or (e) an isolated **polypeptide** having at least 80% sequence identity to S1, lacking its associated signal **peptide**, or to an extracellular domain of S1, with its associated signal **peptide** or lacking its associated signal **peptide**. INDEPENDENT CLAIMS are also included for: (1) an isolated nucleic acid molecule (II) having at least 80% sequence identity to any one of 147 sequences (S3) fully defined in the specification, a nucleotide sequence encoding S1, a full-length coding sequence of S3; a full-length coding sequence of DNA deposited under any ATCC accession number given in the specification; or at least 80% identity to a nucleotide sequence encoding S1, lacking its associated signal **peptide**, a sequence encoding extracellular domain of S1 with or without its associated signal **peptide**; (2) a vector (III) comprising (II); (3) a host cell (IV) comprising (III); (4) preparation of (I); (5) a chimeric molecule (V) comprising (I) fused to a heterologous amino acid sequence; and (6) an antibody (Ab) which specifically binds to (I).

WIDER DISCLOSURE - Disclosed are: (1) a complementary sequence of (II); (2) fragments of (I) and (II), which are useful as hybridization probes; (3) a modified sequence of PRO **polypeptide**; (4) an agonist and antagonist (AA) of (I); (5) a composition comprising (I) or AA; (6) an expressed sequence tag comprising (II), useful as probe; (7) screening assays to identify AA; (8) oligonucleotide probes useful for isolating genomic and cDNA nucleotide sequences or antisense probes, derived from (II); (9) an isolated PRO **polypeptide** which is either transmembrane domain- deleted or transmembrane domain-inactivated; (10) immunoconjugates comprising antibody conjugated to a cytotoxic agent such as chemotherapeutic agent, toxin or radioactive isotope; and (11) an isolated nucleic acid molecule comprising DNA encoding soluble form of (I).

BIOTECHNOLOGY - Preparation: (I) is produced by culturing (IV) under conditions suitable for expression of the PRO **polypeptide** and recovering the PRO polypeptide from the cell culture (claimed). Preferred Nucleic Acid: (II) comprises S3 or its full-length coding sequence. Preferred Vector: (III) is operably linked to control sequences recognized by a host cell transformed with the vector. Preferred Host Cell: (IV) is a Chinese hamster ovary (CHO) cell, Escherichia coli or yeast cell. Preferred Molecule: The heterologous amino acid sequence is an epitope tag sequence or a Fc region of an immunoglobulin. Preferred Antibody: The antibody is a monoclonal antibody, humanized antibody or antibody

fragment.

ACTIVITY - None given.

MECHANISM OF ACTION - Gene therapy; Modulator of cell expressing (I).No supporting data is given.

USE - (I) is useful for detecting PRO943, PRO183, PRO184, PRO185, PRO331, PRO1133, PRO363, PRO5723, PRO1387, PRO1114, PRO3301, PRO9940, PRO1181, PRO7170, PRO361 or PRO846 **polypeptide**, and for linking a bioactive molecule to a cell expressing the above **polypeptides**. The bioactive molecule is a toxin, radiolabel or an antibody. The bioactive material causes the death of the cell. (I) or Ab is useful for modulating at least one biological activity of cell expressing the above **polypeptides** (all claimed). (I) is useful for identifying agonists or antagonists of (I), for preparing variant of (I), as molecular weight markers for protein electrophoresis purpose and (II) is useful for recombinantly expressing those markers. (I) is also useful as therapeutic agent. PRO is useful in assays to identify other proteins or molecules involved in binding interaction. (II) is useful as hybridization probes, in chromosome and gene mapping, in generation of antisense RNA and DNA, in the preparation of PRO polypeptide, for generating transgenic animals or knockout animals which in turn are useful in the development and screening of therapeutically useful reagents, to construct hybridization probes for mapping the gene which encodes the PRO and for the genetic analysis of individuals with genetic disorders, in gene therapy, for chromosome identification, as chromosome marker, and for generating probes for PCR, Northern analysis, Southern analysis and Western analysis. Ab is useful in diagnostic assays for PRO, e.g. detecting its expression in specific cells, tissues or serum, for affinity purification of PRO from recombinant cell culture or natural sources, and for treating septic shock. (I) or Ab is useful for the preparation of medicament for treating conditions which is responsive to the PRO **polypeptide** or anti-PRO antibody. (I) and (II) is useful for tissue typing.

ADMINISTRATION - 10 mug-100 mg/kg, preferably 1 mug-10 mg/kg/day of (I) or Ab is administered through intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, topical, intraarterial or intralesional route, or by **sustained release** system.

EXAMPLE - Isolation of cDNA encoding human PRO281 was as follows. Yeast transformation was performed with limiting amounts of transforming DNA in order to reduce the number of multiple transformed yeast cells. Instead of plasmid isolation from the yeast followed by transformation of Escherichia coli, PCR analysis was performed on single yeast colonies. PCR primers employed were bipartite in order to amplify the insert and a small portion of the invertase gene (allowing to determine that the insert was in frame with invertase) and to add on universal sequencing primer sites. An invertase library was transformed into yeast and positives were selected on **sucrose** plates. Positive clones were re-tested and PCR products were sequenced. The sequence of one clone, PRO281, was determined to contain a signal **peptide** coding sequence. Oligonucleotide primers and probes were designed using the nucleotide sequence of PRO281. A full length plasmid library of cDNAs from human umbilical vein endothelium tissue was titered and approximately 100000 colony forming unit (cfu) were plated. The plates were sealed and were grown overnight. PCR was performed on the individual cultures using primers. Agarose gel electrophoresis was performed and positive wells were identified by visualization of a band of the expected size. Individual positive clones were obtained by colony lift followed by hybridization with (32)P-labeled oligonucleotide. These clones were characterized by PCR, restriction digest, and Southern blot analysis. A full length clone was identified that contained a single open reading frame with an apparent translational initiation site at nucleotide positions 80-82, and a stop signal at nucleotide positions 1115-1117. The predicted **polypeptide** precursor was 345 amino acids long, and had a calculated molecular weight of approximately 37205 daltons and an estimated pI of approximately 10.15. Clone UNQ244 (DNA16422-1209) has been deposited with ATCC on June 2, 1998 and was assigned ATTC deposit number 209929. An analysis of the Dayhoff database using a WU-BLAST- 2 sequence alignment analysis of the full-length

sequence evidenced significant homology between the PRO281 amino acid sequence and the Dayhoff sequences. (640 pages)

L14 ANSWER 13 OF 31 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-20471 BIOTECHDS
TITLE: Novel isolated PRO **polypeptides** e.g., PRO826, PRO1068, PRO1184, PRO1346 and PRO1375, which stimulate proliferation of stimulated T-lymphocytes and are thus therapeutically useful for enhancing immune response;
involving vector-mediated gene transfer and expression in Chinese hamster ovary, Escherichia coli or yeast cell for use in gene therapy
AUTHOR: ASHKENAZI A J; BAKER K P; BOTSTEIN D; DESNOYERS L; EATON D L; FERRARA N; FONG S; GERBER H; GERRITSEN M E; GODDARD A; GODOWSKI P J; GRIMALDI J C; GURNEY A L; KLJAVIN I J; NAPIER M A; PAN J; PAONI N F; ROY M A; STEWART T A; TUMAS D;
WATANABE C K; WILLIAMS P M; WOOD W I; ZHANG Z
PATENT ASSIGNEE: GENENTECH INC
PATENT INFO: US 2003059782 27 Mar 2003
APPLICATION INFO: US 2001-997628 15 Nov 2001
PRIORITY INFO: WO 2001-21735 9 Jul 2001; WO 1997-20069 5 Nov 1997
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-540672 [51]
TI Novel isolated PRO **polypeptides** e.g., PRO826, PRO1068, PRO1184, PRO1346 and PRO1375, which stimulate proliferation of stimulated T-lymphocytes and are thus therapeutically useful for enhancing immune response;
involving vector-mediated gene transfer and expression in Chinese hamster ovary, Escherichia coli or yeast cell for use in gene therapy
AN 2003-20471 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An isolated PRO **polypeptide** (I) having at least 80% sequence identity to: (i) an amino acid sequence chosen from any one of fully defined 147 polypeptide sequences (PS) as given in the specification; or (ii) PS
lacking its associated signal **peptide** or an isolated extracellular domain of PRO **polypeptide** with or without its associated signal **peptide**, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) an isolated nucleic acid (II) having at least 80% identity to: (a) a nucleotide sequence encoding a sequence of PS; (b) a nucleic acid which comprises any one of 147
fully defined PRO polynucleotide sequences (NS) as given in the specification; (c) full-length coding sequence of NS; or (d) nucleotide sequence encoding: (i) PS lacking its associated signal **peptide**; or (ii) an extracellular domain
of PS with or without its associated signal **peptides**; (2) isolated nucleic acid which comprises the full-length coding sequence of DNA deposited under any one of 141 ATCC Accession number, as given in the specification; (3) a vector
(III) comprising (II) having at least 80% sequence identity to nucleotide sequence encoding PS; (4) a host cell (IV) comprising (III); (5) preparation of (I); (6) an isolated PRO **polypeptide** having at least 80% sequence identity to
the amino acid sequence encoded by nucleic acid molecule deposited under any of 141 ATCC accession numbers as described above; (7) a chimeric molecule (V) comprising (I) which has 80% sequence identity to PS, fused to a heterologous amino acid
sequence; (8) an antibody (VI) which specifically binds to (I) which has 80% sequence identity to PS; (9) an isolated extracellular domain (VIII) of the PRO polypeptide; and (10) an isolated PRO **polypeptide** (IX) lacking its
associated signal **peptide**; (11) an isolated **polypeptide** having at least 80% amino acid sequence identity (VIII) or (IX).
WIDER DISCLOSURE - The following are disclosed: (A) complementary nucleic acids; (B) fragments of PRO **polypeptide** coding sequence; (C) a composition comprising PRO **polypeptide**, its agonists or antagonists, anti-PRO
polypeptide useful in treatment of conditions responsive to PRO **polypeptide**, its agonists or antagonists or anti-PRO antibodies; (D) agonists and antagonists of PRO **polypeptides**; (E) variants of (I); and (F)

covalent modifications of (I).

BIOTECHNOLOGY - Preparation: (I) is prepared by standard recombinant techniques (claimed). Preferred Polynucleotide: (II) comprises a full-length coding sequence of NS. Preferred Vector: (III) comprises (II) operably linked to the control sequences recognized by a host cell transformed with the vector. Preferred Host Cell: (IV) is a Chinese hamster ovary cell, yeast cell or an Escherichia coli cell. Preferred Molecule: (V) comprises (I) fused to an epitope tag sequence or to an immunoglobulin Fc region.

ACTIVITY - Vulnerary; Cytostatic; Antiarthritic; Ophthalmological; Nephrotropic.

MECHANISM OF ACTION - PRO antagonist; Stimulates hypertrophy of neonatal heart; Stimulates adrenal cortical capillary endothelial growth; Inhibits vascular endothelial growth factor (VEGF) stimulated proliferation of endothelial cells; Stimulates proliferation of stimulated T-lymphocytes; Enhances survival of rod photoreceptor cells or neuronal cells; Induces proliferation of mammalian kidney mesangial cells; Induces proliferation and/or redifferentiation of chondrocytes in culture. Porcine chondrocytes were isolated by overnight collagenase digestion of articular cartilage of the metacarpophalngeal joint of 4-6 month old female pigs. After 5 days, at 37degreesC, 2 mul of Alamar blue was added to each well and the plates were incubated for an additional 3 hours at 37degreesC. The fluorescence was then measured in each well. The PRO **polypeptide**-treated sample provided a positive result, i.e., a fluorescence more likely that of the positive control than the negative control.

USE - (I) is useful for detecting the PRO **polypeptides** in sample. The sample comprises cells suspected of expressing the PRO **polypeptide**. PRO **polypeptides** which are contacted with the sample are labeled with a detectable label or a solid support. The PRO **polypeptides** are also useful for linking a bioactive molecule to a cell expressing a PRO **polypeptide**, e.g. PRO183, PRO184 or PRO185. The bioactive molecule is a toxin, radiolabel or antibody, which causes the death of the cell. The PRO polypeptides are useful for modulating a biological activity of a cell expressing the PRO **polypeptides**, e.g. the biological activity of a cell expressing PRO183, PRO184 or PRO185 is modulated by contacting the cell with PRO943 polypeptide or anti-PRO183, anti-PRO184 or anti-PRO185 antibody, such that the cell is killed. Similarly, other PRO **polypeptides** are useful for modulating the biological activity of the cell expressing the counterpart polypeptides as described above (all claimed). (II) encoding (I) or its modified forms can also be used to generate either transgenic animals or knockout animals which in turn are useful in the development and screening of therapeutically useful reagents. The PRO **polypeptides** and nucleic acid molecules are useful for tissue typing. The PRO **polypeptides** are also useful as therapeutic agents. PRO1312 stimulates hypertrophy of neonatal heart and is thus useful for treating cardiac insufficiency disorders. PRO1154 and PRO1186 stimulate adrenal cortical capillary endothelial growth, and are thus useful for treating conditions or disorders where angiogenesis would be beneficial, e.g., wound healing, and antibodies against the **polypeptide** are useful for treating cancerous tumors. PRO812 inhibits vascular endothelial growth factor (VEGF) stimulated proliferation of endothelial cells and is thus useful for inhibiting endothelial cell growth in mammals which would be beneficial in inhibiting tumor growth. PRO826, PRO1068, PRO1184, PRO1346 and PRO1375 stimulate proliferation of stimulated T-lymphocytes and are therapeutically useful for enhancing immune response. PRO828, PRO826, PRO1068 or PRO1132 enhance survival of retinal neurons cells and therefore are useful for treating retinal disorders or injuries, e.g. sight loss in mammals due treating retinitis pigmentosum, age-related macular degeneration (AMD). PRO536, PRO943, PRO828, PRO826, PRO1068 or PRO1132 enhance survival/proliferation of rod photoreceptor cells and therefore are useful for treating

retinal disorders of injuries, e.g. sight loss in mammals due to retinitis pigmentosum, AMD. PRO535, PRO826, PRO819, PRO1126, PRO1160 and PRO1387 induce c-fos in endothelial cells, and are thus useful for treating conditions or disorders where angiogenesis would be beneficial, e.g. wound healing and antagonist of this polypeptide (e.g. antibodies against the **polypeptide**) are useful for treating cancerous tumors. PRO819, PRO813 and PRO11066 induce proliferation of mammalian kidney mesangial cells, and therefore are useful for treating kidney disorders associated with decreased mesangial cell function such as Berger disease or other nephropathies associated with dermatitis, herpetiformis or Crohn's disease. PRO1310, PRO844, PRO1312, PRO1192 and PRO1387 induce the proliferation and/or redifferentiation of chondrocytes in culture and are thus useful for treating sports injuries, and arthritis.

ADMINISTRATION - Administered by injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial or intralesional route, topical route or by **sustained release** systems.

The PRO **polypeptide** is administered at a dose of 1 mug/kg-100 mg/kg of mammal body weight or more/day, preferably 1 mug/kg/day-10 mg/kg/day.

EXAMPLE - Isolation of cDNA clones encoding human PRO281 was carried out as follows. In order to obtain a cDNA clone encoding PRO281, methods described in Klein et al., Proc. Natl. Acad. Sci. USA 93:7108-7113 (1996) were employed with the following modifications. Yeast transformation was performed with limiting amounts of transforming DNA in order to reduce the number of multiple transformed yeast cells. Instead of plasmid isolation from the yeast followed by transformation of Escherichia coli. Polymerase chain reaction (PCR) analysis was performed on single yeast colonies. PCR primers employed were bipartite in order to amplify the insert and a small portion of the invertase gene (allowing to determine that the insert was in frame with invertase) and to add on universal sequencing primer sites. An invertase library was transformed into yeast and positives were selected on **sucrose** plates. Positive clones were re-tested and PCR products were sequenced. The sequence of one clone, PRO281, was determined to contain a signal **peptide** coding sequence. Oligonucleotide primers and probes were designed using the nucleotide sequence of PRO281. A full length plasmid library of cDNAs from human umbilical vein endothelium tissue was titered and 100000 cfu (colony forming units) were plated in 192 pools of 500 cfu/pool into 96-well round bottom plates. The plates were sealed and pools were grown overnight at 37degreesC with shaking. PCR was performed on the individual cultures using primers. Agarose gel electrophoresis was performed and positive wells were identified. Individual positive clones were obtained by colony lift followed by hybridization with 32P-labeled oligonucleotide. These clones were characterized by PCR, restriction digest, and southern blot analyses. A full length clone was identified that contained a single open reading frame with an apparent translational initiation site at nucleotide positions 80-82 and a stop signal at nucleotide positions 1115-1117. The predicted **polypeptide** precursor is 345 amino acids (S1) long, has a calculated molecular weight of 37205 Da and an estimated pI of 10.15. Analysis of the full-length PRO281 sequence of (S1) showed the presence of the following: a signal **peptide** from amino acid 1-14, multiple transmembrane domains from amino acids 83-105, 126-146, 158-177, 197-216, 218-238, 245-265, 271-290, and an amino acid sequence block having homology to G-protein coupled receptor proteins from amino acid 115-155. Clone UNQ244 (DNA16422-1209) was deposited with ATCC on Jun 2, 1998 and is assigned ATCC deposit no.209929. An analysis of the Dayhoff database (version 35,45 SwissProt 25), using a WU-BLAST-2 sequence alignment analysis of the full-length sequence of PRO281, evidenced significant homology between the PRO281 amino acid sequence and the following Dayhoff sequences: H64634, AF0330951, B64815, YBHLE-COLI, EMEQUTR1, AF0647633, S53708, A69253, AF03541312 and S63281. (652 pages)

L14 ANSWER 14 OF 31 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-23838 BIOTECHDS

TITLE: Novel isolated secreted and transmembrane PRO **polypeptides** e.g. PRO826, PRO1068, PRO1184, PRO1346 and PRO1375, which stimulate proliferation of stimulated T-lymphocytes and are therapeutically useful for enhancing immune responses;
vector-mediated gene transfer and expression in CHO cell, yeast or bacterium for recombinant protein production for use in disease gene therapy

AUTHOR: ASHKENAZI A J; BAKER K P; BOTSTEIN D; DESNOYERS L; EATON D L; FERRARA N; FONG S; GERBER H; GERRITSEN M E; GODDARD A; GODOWSKI P J; GRIMALDI J C; GURNEY A L; KLJAVIN I J; NAPIER M A; PAN J; PAONI N F; ROY M A; STEWART T A; TUMAS D;
WATANABE C K; WILLIAMS P M; WOOD W I; ZHANG Z
PATENT ASSIGNEE: GENENTECH INC
PATENT INFO: US 2003049682 13 Mar 2003
APPLICATION INFO: US 2001-997573 15 Nov 2001
PRIORITY INFO: WO 2001-21735 9 Jul 2001; WO 1997-20069 5 Nov 1997
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-644678 [61]
TI Novel isolated secreted and transmembrane PRO **polypeptides** e.g. PRO826, PRO1068, PRO1184, PRO1346 and PRO1375, which stimulate proliferation of stimulated T-lymphocytes and are therapeutically useful for enhancing immune responses;
vector-mediated gene transfer and expression in CHO cell, yeast or bacterium for recombinant protein production for use in disease gene therapy
AN 2003-23838 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An isolated secreted and transmembrane PRO **polypeptide** (I) having at least 80% sequence identity to: (i) an amino acid sequence chosen from any one of fully defined 147 **polypeptide** sequences (PS) as given in the specification; or (ii) PS lacking its associated signal **peptide** or an isolated extracellular domain of PRO **polypeptide** with or without its associated signal **peptide**, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) an isolated nucleic acid (II) having at least 80% identity to: (a) a nucleotide sequence encoding a sequence of PS; (b) a nucleic acid which comprises any one of 147 fully defined PRO polynucleotide sequences (NS) as given in the specification; (c) full-length coding sequence of NS; and/or (d) nucleotide sequence encoding: (i) PS lacking its associated signal **peptide**; or (ii) an extracellular domain of PS with or without its associated signal **peptides**; (2) isolated nucleic acid which comprises the full-length coding sequence of DNA deposited under any one of 141 ATCC Accession numbers. as given in the specification; (3) a vector (III) comprising (II) having at least 80% sequence identity to nucleotide sequence encoding PS; (4) a host cell (IV) comprising (III); (5) preparation of (I); (6) an isolated PRO **polypeptide** having at least 80% sequence identity to the amino acid sequence encoded by nucleic acid molecule deposited under any of 141 ATCC accession numbers as described above; (7) a chimeric molecule (V) comprising (I) which has 80% sequence identity to PS, fused to a heterologous amino acid sequence; (8) an antibody (VI) which specifically binds to (I) which has 80% sequence identity to PS; (9) an isolated extracellular domain (VIII) of the PRO **polypeptide**; and (10) an isolated PRO **polypeptide** (IX) lacking its associated signal **peptide**; (11) an isolated polypeptide having at least 80% amino acid sequence identity (VIII) or (IX).
WIDER DISCLOSURE - (1) nucleic acids complementary to above mentioned nucleic acids; (2) fragments of PRO **polypeptide** coding sequence; (3) a composition comprising PRO **polypeptide**, its agonists or antagonists, anti-PRO **polypeptide** useful in treatment of conditions responsive to PRO **polypeptide**, its agonists or antagonists or anti-PRO antibodies; (4) agonists and antagonists of PRO **polypeptides**; (5) variants of (I); and (6) covalent modifications of (I).

BIOTECHNOLOGY - Preparation: (I) is prepared by standard recombinant techniques (claimed). Preferred Polynucleotide: (II) comprises a full-length coding sequence of NS. Preferred Vector: (III) comprises (II) operably linked to the control sequences recognized by a host cell transformed with the vector. Preferred Host Cell: (IV) is a Chinese hamster ovary cell, yeast cell or an Escherichia coli cell. Preferred Molecule: (V) comprises (I) fused to an epitope tag sequence or to an immunoglobulin Fc region.

ACTIVITY - Vulnerary; Cytostatic; Antiarthritic; Ophthalmological; Nephrotropic.

MECHANISM OF ACTION - PRO antagonist; Stimulates hypertrophy of neonatal heart; Stimulates adrenal cortical capillary endothelial growth; Inhibits vascular endothelial growth factor (VEGF) stimulated proliferation of endothelial cells; Stimulates proliferation of stimulated T-lymphocytes; Enhances survival of rod photoreceptor cells or neuronal cells; Induces proliferation of mammalian kidney mesangial cells; Induces proliferation and/or redifferentiation of chondrocytes in culture. The ability of the PRO **polypeptides** PRO1310, PRO844, PRO1312, PRO1192 and PRO1387 to induce the proliferation and/or redifferentiation of chondrocyte in culture was tested in a chondrocyte proliferation assay. Porcine chondrocytes were isolated by overnight collagenase digestion of articular cartilage of the metacarpophalngeal joint of 4-6 month old female pigs. The isolated cells were then seeded at 25000 cells/cm2 in Ham F-12. On day 12, the cells were seeded in 96-well plates at 5000 cells/well in 100 microliters of the media without serum and 100 microliters of either serum-free medium, staurosporin, or the test PRO **polypeptide**. After 5 days, at 37 degreesC, 2 microliters of Alamar blue was added to each well and the plates were incubated for an additional 3 hours at 37 degreesC. The fluorescence was then measured in each well. The PRO **polypeptide**-treated sample provided a positive result, i.e. a fluorescence more likely that of the positive control than the negative control.

USE - The PRO **polypeptides** are useful as molecular weight markers for protein electrophoresis. (I) is also useful for screening compounds to identify those that mimic the PRO **polypeptide** (agonists) or prevent the effect of the PRO **polypeptide** (antagonists). (II) is useful as hybridization probes, in chromosome and gene mapping and in the generation of anti-sense RNA and DNA. PRO nucleic acid is also be useful for the preparation of PRO **polypeptides**. The full-length native sequence of PRO gene or its portions may be used as hybridization probes for a cDNA library to isolate the full-length PRO cDNA or to isolate still other cDNAs. Nucleotide sequences encoding PRO can also be used to construct hybridization probes for mapping the gene which encodes that PRO and for the genetic analysis of individuals with genetic disorders. (II) encoding (I) or its modified forms can also be used to generate either transgenic animals or knockout animals which in turn are useful in the development and screening of therapeutically useful reagents. The PRO **polypeptides** and nucleic acid molecules are useful for tissue typing. The PRO **polypeptides** are also useful as therapeutic agents. PRO1312 stimulates hypertrophy of neonatal heart and is thus useful for treating cardiac insufficiency disorders. PRO1154 and PRO1186 stimulate adrenal cortical capillary endothelial growth, and are thus useful for treating conditions or disorders where angiogenesis would be beneficial, e.g., wound healing, and antibodies against the polypeptide are useful for treating cancerous tumors. PRO812 inhibits vascular endothelial growth factor (VEGF) stimulated proliferation of endothelial cells and is thus useful for inhibiting endothelial cell growth in mammals which would be beneficial in inhibiting tumor growth. PRO826, PRO1068, PRO1184, PRO1346 and PRO1375 stimulate proliferation of stimulated T-lymphocytes and are therapeutically useful for enhancing immune response. PRO828, PRO826, PRO1068 or PRO1132 enhance survival of retinal neurons cells and therefore are useful for treating

retinal disorders or injuries, e.g. sight loss in mammals due treating retinitis pigmentosum, age-related macular degeneration (AMD). PRO536, PRO943, PRO828, PRO826, PRO1068 or PRO1132 enhance survival/proliferation of rod photoreceptor cells

and therefore are useful for treating retinal disorders of injuries, e.g. sight loss in mammals due to retinitis pigmentosum, AMD. PRO535, PRO826, PRO819, PRO1126, PRO1160 and PRO1387 induce c-fos in endothelial cells, and are thus useful for

treating conditions or disorders where angiogenesis would be beneficial, e.g. wound healing and antagonist of this **polypeptide** (e.g. antibodies against the polypeptide) are useful for treating cancerous tumors. PRO819,

PRO813 and PRO11066 induce proliferation of mammalian kidney mesangial cells, and therefore are useful for treating kidney disorders associated with decreased mesangial cell function such as Berger disease or other nephropathies associated

with dermatitis, herpetiformis or Crohn's disease. PRO1310, PRO844, PRO1312, PRO1192 and PRO1387 induce the proliferation and/or redifferentiation of chondrocytes in culture and are thus useful for treating sports injuries, and arthritis.

ADMINISTRATION - Administered by injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial or intralesional route, topical route or by **sustained release** systems.

The PRO **polypeptide** is administered at a dose of 10 ng/kg-100 mg/kg of mammal body weight or more/day, preferably 1 micrograms/kg/day-10 mg/kg/day.

EXAMPLE - Isolation of cDNA clones encoding human PRO281 was carried out as follows. In order to obtain a cDNA clone encoding PRO281, methods described in Klein et al., Proc. Natl. Acad. Sci. USA 93:7108-7113 (1996) were employed with the

following modifications. Yeast transformation was performed with limiting amounts of transforming DNA in order to reduce the number of multiple transformed yeast cells. Instead of plasmid isolation from the yeast followed by transformation of

Escherichia coli. Polymerase chain reaction (PCR) analysis was performed on single yeast colonies. PCR primers employed were bipartite in order to amplify the insert and a small portion of the invertase gene (allowing to determine that the

insert was in frame with invertase) and to add on universal sequencing primer sites. An invertase library was transformed into yeast and positives were selected on **sucrose** plates. Positive clones were re-tested and PCR products were

sequenced. The sequence of one clone, PRO281, was determined to contain a signal **peptide** coding sequence. Oligonucleotide primers and probes were designed using the nucleotide sequence of PRO281. A full length plasmid library of

cDNAs from human umbilical vein endothelium tissue was titered and 100000 cfu (colony forming units) were plated in 192 pools of 500 cfu/pool into 96-well round bottom plates. The plates were sealed and pools were grown overnight at 37

degreesC with shaking. PCR was performed on the individual cultures using primers. Agarose gel electrophoresis was performed and positive wells were identified. Individual positive clones were obtained by colony lift followed by hybridization

with 32P-labeled oligonucleotide. These clones were characterized by PCR, restriction digest, and southern blot analyses. A full length clone was identified that contained a single open reading frame with an apparent translational initiation

site at nucleotide positions 80-82 and a stop signal at nucleotide positions 1115-1117. The predicted **polypeptide** precursor is 345 amino acids (S1) long, has a calculated molecular weight of 37205 Da and an estimated pI of 10.15.

Analysis of the full-length PRO281 sequence of (S1) showed the presence of the following: a signal **peptide** from amino acid 1-14, multiple transmembrane domains from amino acids 83-105, 126-146, 158-177, 197-216, 218-238, 245-265,

271-290, and an amino acid sequence block having homology to G-protein coupled receptor proteins from amino acid 115-155. Clone UNQ244 (DNA16422-1209) was deposited with ATCC on Jun 2, 1998 and is assigned ATCC deposit no.209929. An analysis

of the Dayhoff database (version 35,45 SwissProt 25), using a WU-BLAST-2 sequence alignment analysis of the full-length sequence of PRO281, evidenced significant homology between the PRO281 amino acid sequence and the following Dayhoff

sequences: H64634, AF0330951, B64815, YBHLE-COLI, EMEQUTR1, AF0647633, S53708, A69253, AF03541312 and S63281.(651 pages)

L14 ANSWER 15 OF 31 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-20056 BIOTECHDS
TITLE: New isolated PRO **polypeptides** for example, extracellular, secreted, membrane-bound proteins and receptors useful for stimulating proliferation of T-lymphocytes and enhancing immune responses; recombinant protein production and sense and antisense sequence for use in disease gene therapy
AUTHOR: ASHKENAZI A J; BAKER K P; BOTSTEIN D; DESNOYERS L; EATON D L; FERRARA N; FONG S; GERBER H; GERRITSEN M E; GODDARD A; GODOWSKI P J; GRIMALDI J C; GURNEY A L; KLJAVIN I J; NAPIER M A; PAN J; PAONI N F; ROY M A; STEWART T A; TUMAS D; WATANABE C K; WILLIAMS P M; WOOD W I; ZHANG Z
PATENT ASSIGNEE: GENENTECH INC
PATENT INFO: US 2003049638 13 Mar 2003
APPLICATION INFO: US 2001-991157 16 Nov 2001
PRIORITY INFO: WO 2001-21735 9 Jul 2001; WO 1997-20069 5 Nov 1997
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-521804 [49]
TI New isolated PRO **polypeptides** for example, extracellular, secreted, membrane-bound proteins and receptors useful for stimulating proliferation of T-lymphocytes and enhancing immune responses; recombinant protein production and sense and antisense sequence for use in disease gene therapy
AN 2003-20056 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An isolated PRO **polypeptide** (I) having at least 80% sequence identity to: (a) an amino acid sequence chosen from any one of fully defined 147 polypeptide sequences (PS) as given in the specification; (b) PS lacking its associated signal **peptide**; or (c) an isolated extracellular domain of PRO **polypeptide** with or without its associated signal **peptide**, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) an isolated nucleic acid (II) having at least 80% identity to: (a) a nucleotide sequence encoding a sequence of PS; (b) a nucleic acid which comprises any one of 147 fully defined PRO polynucleotide sequences (NS) as given in the specification; (c) a full-length coding sequence of NS, or (d) a nucleotide sequence encoding: (i) PS lacking its associated signal **peptide**; or (ii) an extracellular domain of PS with or without its associated signal **peptides**; (2) an isolated nucleic acid which comprises the full-length coding sequence of DNA deposited under any one of 141 ATCC Accession numbers given in the specification; (3) a vector (III) comprising (II) having at least 80% sequence identity to a nucleotide sequence encoding PS; (4) a host cell (IV) comprising (III); (5) preparation of (I); (6) an isolated PRO **polypeptide** having at least 80% sequence identity to the amino acid sequence encoded by the nucleic acid molecule deposited under any of 141 ATCC accession numbers as described above; (7) a chimeric molecule (V) comprising (I) which has 80% sequence identity to PS, fused to a heterologous amino acid sequence; (8) an antibody (VI) which specifically binds to (I) which has 80% sequence identity to PS; (9) an isolated extracellular domain (VIII) of the PRO **polypeptide**; and (10) an isolated PRO **polypeptide** (IX) lacking its associated signal **peptide**; and (11) an isolated polypeptide having at least 80% amino acid sequence identity (VIII) or (IX).
WIDER DISCLOSURE - The following are also disclosed as new: (1) nucleic acids complementary to above mentioned nucleic acids; (2) fragments of the PRO polypeptide coding sequence; (3) a composition comprising PRO **polypeptide**, its agonists or antagonists, useful in the treatment of conditions responsive to PRO **polypeptide**, its agonists or antagonists or anti-PRO antibodies; (4) agonists and antagonists of PRO **polypeptides**;

(5) variants of (I); and (6) covalent modifications of (I).

BIOTECHNOLOGY - Preparation: (I) is prepared by standard recombinant techniques (claimed). Preferred Polynucleotide: (II) comprises a full-length coding sequence of NS. Preferred Vector: (III) comprises (II) operably linked to the control sequences recognized by a host cell transformed with the vector. Preferred Host Cell: (IV) is a Chinese hamster ovary cell, yeast cell or an Escherichia coli cell. Preferred Molecule: (V) comprises (I) fused to an epitope tag sequence or to an immunoglobulin Fc region.

ACTIVITY - Vulnerary; Cytostatic; Antiarthritic; Ophthalmological; Nephrotropic; Immunostimulant; Antiinflammatory; Nephrotropic; Osteopathic. The ability of the PRO **polypeptides** PRO1310, PRO844, PRO1312, PRO1192 and PRO1387 to induce the proliferation and/or redifferentiation of chondrocyte in culture was tested in a chondrocyte proliferation assay. Porcine chondrocytes were isolated by overnight collagenase digestion of articular cartilage of the metacarpophalngeal joint of 4-6 month old female pigs. The isolated cells were then seeded at 25000 cells/cm2 in Ham F-12. On day 12, the cells were seeded in 96-well plates at 5000 cells/well in 100 microL of the media without serum and 100 microL of either serum-free medium, staurosporin, or the test PRO **polypeptide**. After 5 days, at 37 degrees C, 2 microL of Alamar blue was added to each well and the plates were incubated for an additional 3 hours at 37 degrees C. The fluorescence was then measured in each well. The PRO **polypeptide**-treated sample provided a positive result, i.e., a fluorescence more like that of the positive control than the negative control.

MECHANISM OF ACTION - PRO antagonist; Vascular Endothelial Growth Factor (VEGF) Inhibitor; T-lymphocyte Stimulator.

USE - (I) is useful for detecting the PRO **polypeptides** in a sample e.g., PRO943 **polypeptide** is detected in a sample suspected of containing a PRO943 polypeptide, by contacting the sample with a PRO183, PRO184 or PRO185 **polypeptide** and determining the formation of PRO943/PRO183, PRO184, or PRO185 **polypeptide** conjugates while the presence of PRO183, PRO184 or PRO185 polypeptide in a sample is detected by contacting them with PRO943 **polypeptide** and determining the formation of the conjugate as described above. The sample comprises cells suspected of expressing the PRO polypeptide. PRO **polypeptides** which are contacted with the sample are labeled with a detectable label or a solid support. The PRO polypeptides are also useful for linking a bioactive molecule to a cell expressing a PRO **polypeptide**. The bioactive molecule is a toxin, radiolabel or antibody, which causes the death of the cell. The PRO **polypeptides** are useful for modulating a biological activity of a cell expressing the PRO **polypeptides**, e.g. the biological activity of a cell expressing PRO183, PRO184 or PRO185 is modulated by contacting the cell with PRO943 **polypeptide** or anti-PRO183, anti-PRO184 or anti-PRO185 antibody, such that the cell is killed. Similarly, other PRO **polypeptides** are useful for modulating the biological activity of the cell expressing the counterpart **polypeptides** as described above (all claimed). The PRO **polypeptides** are useful as molecular weight markers for protein electrophoresis. (I) is also useful for screening compounds to identify those that mimic the PRO **polypeptide** (agonists) or prevent the effect of the PRO **polypeptide** (antagonists). (II) is useful as hybridization probes, in chromosome and gene mapping and in the generation of anti-sense RNA and DNA. (II) encoding (I) or its modified forms can also be used to generate either transgenic animals or knockout animals which in turn are useful in the development and screening of therapeutically useful reagents. The PRO **polypeptides** and nucleic acid molecules are useful for tissue typing. PRO1312 stimulates hypertrophy of neonatal heart and is thus useful for treating cardiac insufficiency disorders. PRO1154 and PRO1186 stimulate adrenal cortical capillary endothelial growth, and are thus useful for treating conditions or disorders where angiogenesis would be beneficial, e.g., wound healing. PRO812 inhibits vascular endothelial growth factor (VEGF) stimulated proliferation of endothelial cells and is thus useful for inhibiting endothelial cell growth in mammals which would be beneficial in inhibiting tumor growth. PRO826, PRO1068, PRO1184, PRO1346 and PRO1375 stimulate proliferation of stimulated

T-lymphocytes and are therapeutically useful for enhancing an immune response. PRO828, PRO826, PRO1068 or PRO1132 enhance survival of retinal neurons cells and therefore are useful for treating retinal disorders or injuries, e.g. sight loss

due to age-related macular degeneration (AMD). PRO535, PRO826, PRO819, PRO1126, PRO1160 and PRO1387 induce c-fos in endothelial cells, and are thus useful for treating conditions or disorders where angiogenesis would be beneficial, e.g. wound

healing. PRO819, PRO813 and PRO11066 induce proliferation of mammalian kidney mesangial cells, and therefore are useful for treating kidney disorders associated with decreased mesangial cell function such as Berger disease or other

nephropathies associated with dermatitis, herpetiformis or Crohn's disease. PRO1310, PRO844, PRO1312, PRO1192 and PRO1387 induce the proliferation and/or redifferentiation of chondrocytes in culture and are thus useful for treating sports

injuries, and arthritis.

ADMINISTRATION - The PRO **polypeptide** is administered at a dose of 10 ng/kg-100 mg/kg of mammal body weight or more/day, preferably 1 microgram/kg/day-10 mg/kg/day. Administration is by injection or infusion by intravenous,

intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial or intralesional routes, topical route or by **sustained release** systems.

EXAMPLE - Isolation of cDNA clones encoding human PRO281 was carried out as follows. In order to obtain a cDNA clone encoding PRO281, methods described in Klein et al., Proc. Natl. Acad. Sci. USA 93:7108-7113 (1996) were employed with the

following modifications. Yeast transformation was performed with limiting amounts of transforming DNA in order to reduce the number of multiple transformed yeast cells instead of plasmid isolation from the yeast followed by transformation of

Escherichia coli. Polymerase chain reaction (PCR) analysis was performed on single yeast colonies. PCR primers employed were bipartite in order to amplify the insert and a small portion of the invertase gene and to add on universal sequencing

primer sites. An invertase library was transformed into yeast and positives were selected on **sucrose** plates. Positive clones were re-tested and PCR products were sequenced. The sequence of one clone, PRO281, was determined to contain

a signal **peptide** coding sequence. Oligonucleotide primers and probes were designed using the nucleotide sequence of PRO281. PCR was performed on a full length plasmid library of cDNAs from human umbilical vein endothelium tissue

using primers. Agarose gel electrophoresis was performed and positive clones were identified. These clones were characterized by PCR, restriction digest, and southern blot analyses. A full length clone was identified that contained a single

open reading frame with an apparent translational initiation site at nucleotide positions 80-82 and a stop signal at nucleotide positions 1115-1117. The predicted **polypeptide** precursor is 345 amino acids (S1) long.(652 pages)

L14 ANSWER 16 OF 31 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-23125 BIOTECHDS
TITLE: Semiconductor device for memory comprises a barrier layer to which inorganic microparticles are distributed with uniform spacing and each particle object pair functions as an electric charge holding area;
fusion protein for memory device bioinformatic hardware
PATENT ASSIGNEE: MATSUSHITA DENKI SANGYO KK
PATENT INFO: JP 2003007871 10 Jan 2003
APPLICATION INFO: JP 2001-194336 27 Jun 2001
PRIORITY INFO: JP 2001-194336 27 Jun 2001; JP 2001-194336 27 Jun 2001
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 2003-629903 [60]
TI Semiconductor device for memory comprises a barrier layer to which inorganic microparticles are distributed with uniform spacing and each particle object pair functions as an electric charge holding area;
fusion protein for memory device bioinformatic hardware
AN 2003-23125 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A barrier layer functioning as an electric charge blocking layer, is formed on a conductor layer on a base plate, where inorganic microparticles (113a,113b) of different sizes are distributed in the barrier layer with uniform distance between them, to function as electric-charge holding area, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for: (1) producing the semiconductor device; (2) fused protein; (3) producing a fused protein; (4) fused protein molecule; and (5) DNA encoding an amino acid sequence of a linker **peptide**.

WIDER DISCLOSURE - The following are disclosed: (a) nucleic acids complementary to above mentioned nucleic acids; (b) nucleic acids having at least 10 nucleotides and produced by hybridizing test DNA molecule under stringent conditions with DNA molecule encoding PRO **polypeptide**, or its complement; (c) fragments of PRO **polypeptide** coding sequence; (d) a composition comprising PRO **polypeptide**, its agonists or antagonists, anti-PRO **polypeptide** useful in treatment of conditions responsive to PRO **polypeptide**, its agonists or antagonists or anti-PRO antibodies; (e) agonists and antagonists of PRO **polypeptides**; (f) variants of (I); and (g) covalent modifications of (I).

BIOTECHNOLOGY - Preparation: (I) is prepared by standard recombinant techniques (claimed). Preferred Polynucleotide: (II) comprises a full-length coding sequence of a sequence chosen from any one of 147 fully defined PRO polynucleotide sequences as given in the specification. Preferred Vector: (III) comprises (II) operably linked to the control sequences recognized by a host cell transformed with the vector. Preferred Host Cell: (IV) is a Chinese hamster ovary cell, yeast cell or an Escherichia coli cell. Preferred Molecule: (V) comprises (I) fused to an epitope tag sequence or to an immunoglobulin Fc region.

ACTIVITY - Vulnerary; Cytostatic; Antiarthritic; Ophthalmological; Nephrotropic.

MECHANISM OF ACTION - Gene therapy; PRO antagonist; Stimulates hypertrophy of neonatal heart; Stimulates adrenal cortical capillary endothelial growth; Inhibits vascular endothelial growth factor (VEGF) stimulated proliferation of endothelial cells; Stimulates proliferation of stimulated T-lymphocytes; Enhances survival of rod photoreceptor cells or neuronal cells; Induces proliferation of mammalian kidney mesangial cells; Induces proliferation and/or redifferentiation of chondrocytes in culture. The ability of the PRO **polypeptides** PRO1310, PRO844, PRO1312, PRO1192 and PRO1387 to induce the proliferation and/or redifferentiation of chondrocyte in culture was tested in a chondrocyte proliferation assay. Porcine chondrocytes were isolated by overnight collagenase digestion of articular cartilage of the metacarpophalangeal joint of 4-6 month old female pigs. The isolated cells were then seeded at 25000 cells/cm2 in Ham F-12. On day 12, the cells were seeded in 96-well plates at 5000 cells/well in 100microl of the media without serum and 100 microl of either serum-free medium, staurosporin, or the test PRO **polypeptide**. After 5 days, at 37degreesC, 2 microl of Alamar blue was added to each well and the plates were incubated for an additional 3 hours at 37degreesC. The fluorescence was then measured in each well. The PRO polypeptide-treated sample provided a positive result, i.e., a fluorescence more likely that of the positive control than the negative control.

USE - (I) is useful for detecting the PRO **polypeptides** in a sample e.g., PRO943 **polypeptide** is detected in a sample suspected of containing a PRO943 polypeptide, by contacting the sample with a PRO183, PRO184 or PRO185 **polypeptide** and determining the formation of PRO943/PRO183, PRO184, or PRO185 **polypeptide** conjugate; and the presence of PRO183, PRO184 or PRO185 polypeptide in a sample is detected by contacting PRO943 **polypeptide** and determining the formation of the conjugate as described above. Similarly, the **polypeptides** PRO331; PRO363 or PRO5723; PRO1114; PRO1181 are detected by contacting sample with PRO1133; PRO1387; PRO3301 or PRO9940; PRO7170, PRO361 or PRO846, **polypeptides**, respectively and determining formation of conjugate. And for detecting PRO1133; PRO1387; PRO3301 or PRO9940; PRO7170, PRO361 or PRO846, **polypeptides**, the sample is contacted

with PRO331; PRO363 or PRO5723; PRO1114; PRO1181 **polypeptides**, respectively, and formation of conjugate is determined. The sample comprises cells suspected of expressing the PRO **polypeptide**. PRO **polypeptides** which are contacted with the sample are labeled with a detectable label or a solid support. The PRO **polypeptides** are also useful for linking a bioactive molecule to a cell expressing a PRO **polypeptide**, e.g. PRO183, PRO184 or PRO185; PRO1133; PRO363 or PRO5723; PRO3301 or PRO9940; PRO7170, PRO361 or PRO846, **polypeptides** are useful for linking a bioactive molecule to a cell expressing PRO943; PRO331; PRO1387; PRO1114; PRO1181 **polypeptides**, respectively, and the **polypeptides** PRO943; PRO331; PRO1387; PRO1114; PRO1181 are useful for linking a bioactive molecule to a cell expressing PRO183, PRO184 or PRO185; PRO1133; PRO363 or PRO5723; PRO3301 or PRO9940; PRO7170, PRO361 or PRO846, **polypeptides**, respectively. The bioactive molecule is a toxin, radiolabel or antibody, which causes the death of the cell. The PRO **polypeptides** are useful for modulating a biological activity of a cell expressing the PRO **polypeptides**, e.g. the biological activity of a cell expressing PRO183, PRO184 or PRO185 is modulated by contacting the cell with PRO943 **polypeptide** or anti-PRO183, anti-PRO184 or anti-PRO185 antibody, such that the cell is killed. Similarly, other PRO **polypeptides** are useful for modulating the biological activity of the cell expressing the counterpart **polypeptides** as described above (all claimed). The PRO **polypeptides** are also useful as therapeutic agents e.g. treating cardiac insufficiency disorders, disorders where angiogenesis would be beneficial, e.g., wound healing, inhibiting tumor growth, enhancing immune response, treating retinal disorders or injuries, treating kidney disorders associated with decreased mesangial cell function such as Berger disease or other nephropathies associated with dermatitis, herpetiformis or Crohn's disease, and treating sports injuries, and arthritis.

ADMINISTRATION - Administered by injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial or intralesional route, topical route or by **sustained release** systems.

The PRO **polypeptide** is administered at a dose of 10 microg/kg-100 mg/kg of mammal body weight or more/day, preferably 1 microg/kg/day-10 mg/kg/day.

ADVANTAGE - The semiconductor device has high reliability.

EXAMPLE - Isolation of cDNA clones encoding human PRO281 was carried out as follows. In order to obtain a cDNA clone encoding PRO281, methods described in Klein et al., Proc. Natl. Acad. Sci. USA 93:7108-7113 (1996) were employed with the following modifications. Yeast transformation was performed with limiting amounts of transforming DNA in order to reduce the number of multiple transformed yeast cells. Instead of plasmid isolation from the yeast followed by transformation of Escherichia coli. Polymerase chain reaction (PCR) analysis was performed on single yeast colonies. PCR primers employed were bipartite in order to amplify the insert and a small portion of the invertase gene (allowing to determine that the insert was in frame with invertase) and to add on universal sequencing primer sites. An invertase library was transformed into yeast and positives were selected on **sucrose** plates. Positive clones were re-tested and PCR products were sequenced. The sequence of one clone, PRO281, was determined to contain a signal **peptide** coding sequence. Oligonucleotide primers and probes were designed using the nucleotide sequence of PRO281. A full length plasmid library of cDNAs from human umbilical vein endothelium tissue was titered and 100000 cfu (colony forming units) were plated in 192 pools of 500 cfu/pool into 96-well round bottom plates. The plates were sealed and pools were grown overnight at 37degreesC with shaking. PCR was performed on the individual cultures using primers. Agarose gel electrophoresis was performed and positive wells were identified. Individual positive clones were obtained by colony lift followed by hybridization with 32P-labeled oligonucleotide. These clones were characterized by PCR, restriction digest, and southern blot analyses. A full length clone was identified that contained a single open reading frame with an apparent translational initiation site at nucleotide positions 80-82 and a stop signal at nucleotide positions 1115-1117. The predicted **polypeptide** precursor is 345 amino acids (S1) long, has a calculated molecular weight of 37205 Da and an estimated pI of 10.15. Analysis

of the full-length PRO281 sequence of (S1) showed the presence of the following: a signal **peptide** from amino acid 1-14, multiple transmembrane domains from amino acids 83-105, 126-146, 158-177, 197-216, 218-238, 245-265, 271-290, and an amino acid sequence block having homology to G-protein coupled receptor proteins from amino acid 115-155. Clone UNQ244 (DNA16422-1209) was deposited with ATCC on Jun 2, 1998 and is assigned ATCC deposit no.209929. An analysis of the Dayhoff database (version 35,45 SwissProt 25), using a WU-BLAST-2 sequence alignment analysis of the full-length sequence of PRO281, evidenced significant homology between the PRO281 amino acid sequence and the following Dayhoff sequences: H64634, AF0330951, B64815, YBHLE-COLI, EMEQUTR1, AF0647633, S53708, A69253, AF03541312 and S63281. (16 pages)

L14 ANSWER 17 OF 31 PHIN COPYRIGHT 2005 PJB on STN

ACCESSION NUMBER: 2002:22443 PHIN
DOCUMENT NUMBER: W00780366
DATA ENTRY DATE: 1 Dec 2002
TITLE: Durect/Endo sign Chronogesic deal, design changes delay (Keywords - IMPLANTS/CONTROLLED RELEASE)
SOURCE: Target (2002) No. 12 p15
DOCUMENT TYPE: Newsletter
FILE SEGMENT: FULL
TI Durect/Endo sign Chronogesic deal, design changes delay (Keywords - IMPLANTS/CONTROLLED RELEASE)

L14 ANSWER 18 OF 31 PHIN COPYRIGHT 2005 PJB on STN

ACCESSION NUMBER: 2002:2188 PHIN
DOCUMENT NUMBER: W00738712
DATA ENTRY DATE: 1 Jan 2002
TITLE: October patent applications
SOURCE: Target (2002) No. 1 November 2001 Special Issue p6
DOCUMENT TYPE: Newsletter
FILE SEGMENT: FULL
TI October patent applications

L14 ANSWER 19 OF 31 TOXCENTER COPYRIGHT 2005 ACS on STN DUPLICATE 1
ACCESSION NUMBER: 2002:262489 TOXCENTER
COPYRIGHT: Copyright 2005 ACS
DOCUMENT NUMBER: CA13724358114J
TITLE: **Peptide**-containing preparations
AUTHOR(S): Takada, Shigeyuki; Ohtaki, Tetsuya; Omachi, Yoshihiro; Yamada, Takao
CORPORATE SOURCE: ASSIGNEE: Takeda Chemical Industries, Ltd.
PATENT INFORMATION: WO 2002085399 A1 31 Oct 2002
SOURCE: (2002) PCT Int. Appl., 47 pp.
CODEN: PIXXD2.
COUNTRY: JAPAN
DOCUMENT TYPE: Patent
FILE SEGMENT: CAPLUS
OTHER SOURCE: CAPLUS 2002:832642
LANGUAGE: Japanese
ENTRY DATE: Entered STN: 20021112
Last Updated on STN: 20021210
TI **Peptide**-containing preparations
AB Disclosed are metastin-containing prepns. which have an activity of inhibiting cancer metastasis, are useful in treating or preventing any cancers, have an effect of controlling placental functions, are useful in treating or preventing villus cancer, hydatid mole, invasive mole, abortion, fetal hypoplasia, sugar metabolic error, lipid metabolic error or abnormalities in delivery, can exert the drug effect only in a small dose, can relieve side effects, are not necessarily

administered everyday, and can relieve inconvenience and pain of patients. Because of having an activity of inhibiting cancer metastasis, these prepns. are particularly useful in treating or preventing any cancers. Because of having an effect

of controlling placental functions, these prepns. are useful in treating or preventing villus cancer, hydatid mole, invasive mole, abortion, fetal hypoplasia, sugar metabolic error, lipid metabolic error or abnormalities in delivery. Because

of having an effect of controlling pancreatic functions, the prepns. are also useful in treating or preventing pancreatic diseases. For example, **sustained**-release microcapsules containing metastin, glycolic acid-lactic acid

copolymer, and **mannitol** were prepared

L14 ANSWER 20 OF 31 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-23853 BIOTECHDS
TITLE: Novel isolated PRO **polypeptides** e.g., PRO826, PRO1068, PRO1184, PRO1346 and PRO1375, which stimulate proliferation of stimulated T-lymphocytes and are thus therapeutically useful for enhancing immune response;
involving vector-mediated gene transfer and expression in Chinese hamster ovary, yeast or Escherichia coli for use in gene therapy
AUTHOR: ASHKENAZI A J; BAKER K P; BOTSTEIN D; DESNOYERS L; EATON D L; FERRARA N; FONG S; GERBER H; GERRITSEN M E; GODDARD A; GODOWSKI P J; GRIMALDI J C; GURNEY A L; KLJAVIN I J; NAPIER M A; PAN J; PAONI N F; ROY M A; STEWART T A; TUMAS D;
WATANABE C K; WILLIAMS P M; WOOD W I; ZHANG Z
PATENT ASSIGNEE: GENENTECH INC
PATENT INFO: US 2002193300 19 Dec 2002
APPLICATION INFO: US 2001-990444 14 Nov 2001
PRIORITY INFO: WO 2001-21735 9 Jul 2001; WO 1997-20069 5 Nov 1997
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-657231 [62]
TI Novel isolated PRO **polypeptides** e.g., PRO826, PRO1068, PRO1184, PRO1346 and PRO1375, which stimulate proliferation of stimulated T-lymphocytes and are thus therapeutically useful for enhancing immune response;
involving vector-mediated gene transfer and expression in Chinese hamster ovary, yeast or Escherichia coli for use in gene therapy
AN 2003-23853 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An isolated PRO **polypeptide** (I) having at least 80% sequence identity to: (i) an amino acid sequence chosen from any one of fully defined 147 polypeptide sequences (PS) as given in the specification; or (ii) PS

lacking its associated signal **peptide** or an isolated extracellular domain of PRO **polypeptide** with or without its associated signal **peptide**, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid (II) having at least 80% identity to a nucleotide sequence encoding (I); (2) an isolated nucleic acid comprising the full-length

coding sequence of any one of 141 DNA sequences deposited under ATCC Accession Number as given in the specification; (3) a vector (III) comprising (II); (4) a host cell (IV) comprising (III); (5) preparation of (I); (6) an isolated PRO

polypeptide having at least 80% sequence identity to the amino acid sequence encoded by nucleic acid molecule deposited under any of ATCC accession number as described above; (7) a chimeric molecule (V) comprising (I) fused to a

heterologous amino acid sequence; (8) an antibody (VI) which specifically binds to (I); (9) an isolated nucleic acid molecule (VII) comprising 80% sequence identity to: (a) a nucleic acid comprising a sequence chosen from any one of 147 fully

defined PRO polynucleotide sequences (NS) as given in the specification; or (b) the full-length coding sequence of NS; (10) an isolated extracellular domain (VIII) of the PRO **polypeptide**; (11) an isolated PRO **polypeptide**

(IX) lacking its associated signal **peptide**; and (12) an isolated **polypeptide** having at least 80% amino acid sequence identity (VIII) or (IX).

WIDER DISCLOSURE - The following are disclosed: (a) nucleic acids complementary to above mentioned nucleic acids; (b) nucleic acids having at least 10 nucleotides and produced by hybridizing test DNA molecule under stringent conditions with DNA molecule encoding PRO **polypeptide**, or its complement; (c) fragments of PRO **polypeptide** coding sequence; (d) a composition comprising PRO **polypeptide**, its agonists or antagonists, anti-PRO **polypeptide** useful in treatment of conditions responsive to PRO **polypeptide**, its agonists or antagonists or anti-PRO antibodies; (e) agonists and antagonists of PRO **polypeptides**; (f) variants of (I); and (g) covalent modifications of (I).

BIOTECHNOLOGY - Preparation: (I) is prepared by standard recombinant techniques (claimed). Preferred Polynucleotide: (II) comprises a full-length coding sequence of a sequence chosen from any one of 147 fully defined PRO polynucleotide sequences as given in the specification. Preferred Vector: (III) comprises (II) operably linked to the control sequences recognized by a host cell transformed with the vector. Preferred Host Cell: (IV) is a Chinese hamster ovary cell, yeast cell or an Escherichia coli cell. Preferred Molecule: (V) comprises (I) fused to an epitope tag sequence or to an immunoglobulin Fc region.

ACTIVITY - Vulnerary; Cytostatic; Antiarthritic; Ophthalmological; Nephrotropic.

MECHANISM OF ACTION - Gene therapy; PRO antagonist; Stimulates hypertrophy of neonatal heart; Stimulates adrenal cortical capillary endothelial growth; Inhibits vascular endothelial growth factor (VEGF) stimulated proliferation of endothelial cells; Stimulates proliferation of stimulated T-lymphocytes; Enhances survival of rod photoreceptor cells or neuronal cells; Induces proliferation of mammalian kidney mesangial cells; Induces proliferation and/or redifferentiation of chondrocytes in culture. The ability of the PRO **polypeptides** PRO1310, PRO844, PRO1312, PRO1192 and PRO1387 to induce the proliferation and/or redifferentiation of chondrocyte in culture was tested in a chondrocyte proliferation assay. Porcine chondrocytes were isolated by overnight collagenase digestion of articular cartilage of the metacarpophalangeal joint of 4-6 month old female pigs. The isolated cells were then seeded at 25000 cells/cm2 in Ham F-12. On day 12, the cells were seeded in 96-well plates at 5000 cells/well in 100microl of the media without serum and 100 microl of either serum-free medium, staurosporin, or the test PRO **polypeptide**. After 5 days, at 37degreesC, 2 microl of Alamar blue was added to each well and the plates were incubated for an additional 3 hours at 37degreesC. The fluorescence was then measured in each well. The PRO polypeptide-treated sample provided a positive result, i.e., a fluorescence more likely that of the positive control than the negative control.

USE - (I) is useful for detecting the PRO **polypeptides** in sample e.g., PRO943 **polypeptide** is detected in a sample suspected of containing a PRO943 polypeptide, by contacting the sample with a PRO183, PRO184 or PRO185 **polypeptide** and determining the formation of PRO943/PRO183, PRO184, or PRO185 **polypeptide** conjugate; and the presence of PRO183, PRO184 or PRO185 polypeptide in a sample is detected by contacting PRO943 **polypeptide** and determining the formation of the conjugate as described above. Similarly, the **polypeptides** PRO331; PRO363 or PRO5723; PRO1114; PRO1181 are detected by contacting sample with PRO1133; PRO1387; PRO3301 or PRO9940; PRO7170, PRO361 or PRO846, **polypeptides**, respectively and determining formation of conjugate. And for detecting PRO1133; PRO1387; PRO3301 or PRO9940; PRO7170, PRO361 or PRO846, **polypeptides**, the sample is contacted with PRO331; PRO363 or PRO5723; PRO1114; PRO1181 **polypeptides**, respectively, and formation of conjugate is determined. The sample comprises cells suspected of expressing the PRO **polypeptide**. PRO **polypeptides** which are contacted with the sample are labeled with a detectable label or a solid support. The PRO **polypeptides** are also useful for linking a bioactive molecule to a cell expressing a PRO **polypeptide**, e.g. PRO183, PRO184 or PRO185; PRO1133; PRO363 or PRO5723; PRO3301 or PRO9940; PRO7170, PRO361 or PRO846, **polypeptides** are useful for linking a bioactive molecule to a cell expressing PRO943; PRO331; PRO1387; PRO1114; PRO1181 **polypeptides**,

respectively, and the **polypeptides** PRO943; PRO331; PRO1387; PRO1114; PRO1181 are useful for linking a bioactive molecule to a cell expressing PRO183, PRO184 or PRO185; PRO1133; PRO363 or PRO5723; PRO3301 or PRO9940; PRO7170, PRO361 or PRO846, **polypeptides**, respectively. The bioactive molecule is a toxin, radiolabel or antibody, which causes the death of the cell. The PRO **polypeptides** are useful for modulating a biological activity of a cell expressing the PRO **polypeptides**, e.g. the biological activity of a cell expressing PRO183, PRO184 or PRO185 is modulated by contacting the cell with PRO943 **polypeptide** or anti-PRO183, anti-PRO184 or anti-PRO185 antibody, such that the cell is killed. Similarly, other PRO **polypeptides** are useful for modulating the biological activity of the cell expressing the counterpart **polypeptides** as described above (all claimed). The PRO **polypeptides** are also useful as therapeutic agents e.g treating cardiac insufficiency disorders, conditions or disorders where angiogenesis would be beneficial, e.g., wound healing and tumors, enhancing immune response, treating retinal disorders or injuries, treating kidney disorders associated with decreased mesangial cell function such as Berger disease or other nephropathies associated with dermatitis, herpetiformis or Crohn's disease, and treating sports injuries, and arthritis.

ADMINISTRATION - Administered by injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial or intralesional route, topical route or by **sustained release** systems.

The PRO **polypeptide** is administered at a dose of 10 microg/kg-100 mg/kg of mammal body weight or more/day, preferably 1 microg/kg/day-10 mg/kg/day.

EXAMPLE - Isolation of cDNA clones encoding human PRO281 was carried out as follows. In order to obtain a cDNA clone encoding PRO281, methods described in Klein et al., Proc. Natl. Acad. Sci. USA 93:7108-7113 (1996) were employed with the following modifications. Yeast transformation was performed with limiting amounts of transforming DNA in order to reduce the number of multiple transformed yeast cells. Instead of plasmid isolation from the yeast followed by transformation of Escherichia coli. Polymerase chain reaction (PCR) analysis was performed on single yeast colonies. PCR primers employed were bipartite in order to amplify the insert and a small portion of the invertase gene (allowing to determine that the insert was in frame with invertase) and to add on universal sequencing primer sites. An invertase library was transformed into yeast and positives were selected on **sucrose** plates. Positive clones were re-tested and PCR products were sequenced. The sequence of one clone, PRO281, was determined to contain a signal **peptide** coding sequence. Oligonucleotide primers and probes were designed using the nucleotide sequence of PRO281. A full length plasmid library of cDNAs from human umbilical vein endothelium tissue was titered and 100000 cfu (colony forming units) were plated in 192 pools of 500 cfu/pool into 96-well round bottom plates. The plates were sealed and pools were grown overnight at 37degreesC with shaking. PCR was performed on the individual cultures using primers. Agarose gel electrophoresis was performed and positive wells were identified. Individual positive clones were obtained by colony lift followed by hybridization with 32P-labeled oligonucleotide. These clones were characterized by PCR, restriction digest, and southern blot analyses. A full length clone was identified that contained a single open reading frame with an apparent translational initiation site at nucleotide positions 80-82 and a stop signal at nucleotide positions 1115-1117. The predicted **polypeptide** precursor is 345 amino acids (S1) long, has a calculated molecular weight of 37205 Da and an estimated pI of 10.15. Analysis of the full-length PRO281 sequence of (S1) showed the presence of the following: a signal **peptide** from amino acid 1-14, multiple transmembrane domains from amino acids 83-105, 126-146, 158-177, 197-216, 218-238, 245-265, 271-290, and an amino acid sequence block having homology to G-protein coupled receptor proteins from amino acid 115-155. Clone UNQ244 (DNA16422-1209) was deposited with ATCC on Jun 2, 1998 and is assigned ATCC deposit no.209929. An analysis of the Dayhoff database (version 35,45 SwissProt 25), using a WU-BLAST-2 sequence alignment analysis of the full-length sequence of PRO281, evidenced significant homology between the PRO281 amino acid sequence and the following Dayhoff sequences: H64634,

AF0330951, B64815, YBHLE-COLI, EMEQUTR1, AF0647633, S53708, A69253, AF03541312 and S63281. (653 pages)

L14 ANSWER 21 OF 31 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-23852 BIOTECHDS
TITLE: Isolated PRO **polypeptides** e.g., PRO826, PRO1068, PRO1184, PRO1346 and PRO1375, which stimulate proliferation of stimulated T-lymphocytes and are thus therapeutically useful e.g. for enhancing immune response;
involving vector-mediated gene transfer and expression in Chinese hamster ovary, yeast or Escherichia coli for use in gene therapy
AUTHOR: ASHKENAZI A J; BAKER K P; BOTSTEIN D; DESNOYERS L; EATON D L; FERRARA N; FONG S; GERBER H; GERRITSEN M E; GODDARD A; GODOWSKI P J; GRIMALDI J C; GURNEY A L; KLJAVIN I J; NAPIER M A; PAN J; PAONI N F; ROY M A; STEWART T A; TUMAS D;
WATANABE C K; WILLIAMS P M; WOOD W I; ZHANG Z
PATENT ASSIGNEE: GENENTECH INC
PATENT INFO: US 2002193299 19 Dec 2002
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TI Isolated PRO **polypeptides** e.g., PRO826, PRO1068, PRO1184, PRO1346 and PRO1375, which stimulate proliferation of stimulated T-lymphocytes and are thus therapeutically useful e.g. for enhancing immune response;
involving vector-mediated gene transfer and expression in Chinese hamster ovary, yeast or Escherichia coli for use in gene therapy
AN 2003-23852 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An isolated PRO **polypeptide** (I) having at least 80% sequence identity to: (i) an amino acid sequence chosen from any one of fully defined 147 polypeptide sequences (PS) as given in the specification; or (ii) PS
lacking its associated signal **peptide** or an isolated extracellular domain of PRO **polypeptide** with or without its associated signal **peptide**, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid (II) having at least 80% identity to a nucleotide sequence encoding (I); (2) an isolated nucleic acid comprising the full-length
coding sequence of any one of 141 DNA sequences deposited under ATCC Accession Number as given in the specification; (3) a vector (III) comprising (II); (4) a host cell (IV) comprising (III); (5) preparation of (I); (6) an isolated PRO
polypeptide having at least 80% sequence identity to the amino acid sequence encoded by nucleic acid molecule deposited under any of ATCC accession number as described above; (7) a chimeric molecule (V) comprising (I) fused to a
heterologous amino acid sequence; (8) an antibody (VI) which specifically binds to (I); (9) an isolated nucleic acid molecule (VII) comprising 80% sequence identity to: (a) a nucleic acid comprising a sequence chosen from any one of 147 fully
defined PRO polynucleotide sequences (NS) as given in the specification; or (b) the full-length coding sequence of NS; (10) an isolated extracellular domain (VIII) of the PRO **polypeptide**; (11) an isolated PRO **polypeptide**
(IX) lacking its associated signal **peptide**; and (12) an isolated **polypeptide** having at least 80% amino acid sequence identity (VIII) or (IX).
WIDER DISCLOSURE - The following are disclosed: (a) nucleic acids complementary to above mentioned nucleic acids; (b) nucleic acids having at least 10 nucleotides and produced by hybridizing test DNA molecule under stringent conditions
with DNA molecule encoding PRO **polypeptide**, or its complement; (c) fragments of PRO **polypeptide** coding sequence; (d) a composition comprising PRO **polypeptide**, its agonists or antagonists, anti-PRO
polypeptide useful in treatment of conditions responsive to PRO **polypeptide**, its agonists or antagonists or anti-PRO antibodies; (e) agonists and antagonists of PRO **polypeptides**; (f) variants of (I); and (g)

covalent modifications of (I).

BIOTECHNOLOGY - Preparation: (I) is prepared by standard recombinant techniques (claimed). Preferred Polynucleotide: (II) comprises a full-length coding sequence of a sequence chosen from any one of 147 fully defined PRO polynucleotide sequences as given in the specification. Preferred Vector: (III) comprises (II) operably linked to the control sequences recognized by a host cell transformed with the vector. Preferred Host Cell: (IV) is a Chinese hamster ovary cell, yeast cell or an Escherichia coli cell. Preferred Molecule: (V) comprises (I) fused to an epitope tag sequence or to an immunoglobulin Fc region.

ACTIVITY - Vulnerary; Cytostatic; Antiarthritic; Ophthalmological; Nephrotropic.

MECHANISM OF ACTION - Gene therapy; PRO antagonist; Stimulates hypertrophy of neonatal heart; Stimulates adrenal cortical capillary endothelial growth; Inhibits vascular endothelial growth factor (VEGF) stimulated proliferation of endothelial cells; Stimulates proliferation of stimulated T-lymphocytes; Enhances survival of rod photoreceptor cells or neuronal cells; Induces proliferation of mammalian kidney mesangial cells; Induces proliferation and/or redifferentiation of chondrocytes in culture. The ability of the PRO **polypeptides** PRO1310, PRO844, PRO1312, PRO1192 and PRO1387 to induce the proliferation and/or redifferentiation of chondrocyte in culture was tested in a chondrocyte proliferation assay. Porcine chondrocytes were isolated by overnight collagenase digestion of articular cartilage of the metacarpophalangeal joint of 4-6 month old female pigs. The isolated cells were then seeded at 25000 cells/cm2 in Ham F-12. On day 12, the cells were seeded in 96-well plates at 5000 cells/well in 100microl of the media without serum and 100 microl of either serum-free medium, staurosporin, or the test PRO **polypeptide**. After 5 days, at 37degreesC, 2 microl of Alamar blue was added to each well and the plates were incubated for an additional 3 hours at 37degreesC. The fluorescence was then measured in each well. The PRO polypeptide-treated sample provided a positive result, i.e., a fluorescence more likely that of the positive control than the negative control.

USE - (I) is useful for detecting the PRO **polypeptides** in a sample e.g., PRO943 **polypeptide** is detected in a sample suspected of containing a PRO943 polypeptide, by contacting the sample with a PRO183, PRO184 or PRO185 **polypeptide** and determining the formation of PRO943/PRO183, PRO184, or PRO185 **polypeptide** conjugate; and the presence of PRO183, PRO184 or PRO185 polypeptide in a sample is detected by contacting PRO943 **polypeptide** and determining the formation of the conjugate as described above. Similarly, the **polypeptides** PRO331; PRO363 or PRO5723; PRO1114; PRO1181 are detected by contacting sample with PRO1133; PRO1387; PRO3301 or PRO9940; PRO7170, PRO361 or PRO846, **polypeptides**, respectively and determining formation of conjugate. And for detecting PRO1133; PRO1387; PRO3301 or PRO9940; PRO7170, PRO361 or PRO846, **polypeptides**, the sample is contacted with PRO331; PRO363 or PRO5723; PRO1114; PRO1181 **polypeptides**, respectively, and formation of conjugate is determined. The sample comprises cells suspected of expressing the PRO **polypeptide**. PRO **polypeptides** which are contacted with the sample are labeled with a detectable label or a solid support. The PRO **polypeptides** are also useful for linking a bioactive molecule to a cell expressing a PRO **polypeptide**, e.g. PRO183, PRO184 or PRO185; PRO1133; PRO363 or PRO5723; PRO3301 or PRO9940; PRO7170, PRO361 or PRO846, **polypeptides** are useful for linking a bioactive molecule to a cell expressing PRO943; PRO331; PRO1387; PRO1114; PRO1181 **polypeptides**, respectively, and the **polypeptides** PRO943; PRO331; PRO1387; PRO1114; PRO1181 are useful for linking a bioactive molecule to a cell expressing PRO183, PRO184 or PRO185; PRO1133; PRO363 or PRO5723; PRO3301 or PRO9940; PRO7170, PRO361 or PRO846, **polypeptides**, respectively. The bioactive molecule is a toxin, radiolabel or antibody, which causes the death of the cell. The PRO **polypeptides** are useful for modulating a biological activity of a cell expressing the PRO **polypeptides**, e.g. the biological activity of a cell expressing PRO183, PRO184 or PRO185 is modulated by contacting the cell with PRO943 **polypeptide** or anti-PRO183, anti-PRO184 or anti-PRO185 antibody, such that the

cell is killed. Similarly, other PRO **polypeptides** are useful for modulating the biological activity of the cell expressing the counterpart **polypeptides** as described above (all claimed). The PRO **polypeptides** are also useful as therapeutic agents e.g. treating cardiac insufficiency disorders, disorders where angiogenesis would be beneficial, e.g., wound healing, inhibiting tumor growth, enhancing immune response, treating retinal disorders or injuries, treating kidney disorders associated with decreased mesangial cell function such as Berger disease or other nephropathies associated with dermatitis, herpetiformis or Crohn's disease, and treating sports injuries, and arthritis.

ADMINISTRATION - Administered by injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial or intralesional route, topical route or by **sustained release** systems.

The PRO **polypeptide** is administered at a dose of 10 microg/kg-100 mg/kg of mammal body weight or more/day, preferably 1 microg/kg/day-10 mg/kg/day.

EXAMPLE - Isolation of cDNA clones encoding human PRO281 was carried out as follows. In order to obtain a cDNA clone encoding PRO281, methods described in Klein et al., Proc. Natl. Acad. Sci. USA 93:7108-7113 (1996) were employed with the following modifications. Yeast transformation was performed with limiting amounts of transforming DNA in order to reduce the number of multiple transformed yeast cells. Instead of plasmid isolation from the yeast followed by transformation of Escherichia coli. Polymerase chain reaction (PCR) analysis was performed on single yeast colonies. PCR primers employed were bipartite in order to amplify the insert and a small portion of the invertase gene (allowing to determine that the insert was in frame with invertase) and to add on universal sequencing primer sites. An invertase library was transformed into yeast and positives were selected on **sucrose** plates. Positive clones were re-tested and PCR products were sequenced. The sequence of one clone, PRO281, was determined to contain a signal **peptide** coding sequence. Oligonucleotide primers and probes were designed using the nucleotide sequence of PRO281. A full length plasmid library of cDNAs from human umbilical vein endothelium tissue was titered and 100000 cfu (colony forming units) were plated in 192 pools of 500 cfu/pool into 96-well round bottom plates. The plates were sealed and pools were grown overnight at 37degreesC with shaking. PCR was performed on the individual cultures using primers. Agarose gel electrophoresis was performed and positive wells were identified. Individual positive clones were obtained by colony lift followed by hybridization with 32P-labeled oligonucleotide. These clones were characterized by PCR, restriction digest, and southern blot analyses. A full length clone was identified that contained a single open reading frame with an apparent translational initiation site at nucleotide positions 80-82 and a stop signal at nucleotide positions 1115-1117. The predicted **polypeptide** precursor is 345 amino acids (S1) long, has a calculated molecular weight of 37205 Da and an estimated pI of 10.15. Analysis of the full-length PRO281 sequence of (S1) showed the presence of the following: a signal **peptide** from amino acid 1-14, multiple transmembrane domains from amino acids 83-105, 126-146, 158-177, 197-216, 218-238, 245-265, 271-290, and an amino acid sequence block having homology to G-protein coupled receptor proteins from amino acid 115-155. Clone UNQ244 (DNA16422-1209) was deposited with ATCC on Jun 2, 1998 and is assigned ATCC deposit no.209929. An analysis of the Dayhoff database (version 35,45 SwissProt 25), using a WU-BLAST-2 sequence alignment analysis of the full-length sequence of PRO281, evidenced significant homology between the PRO281 amino acid sequence and the following Dayhoff sequences: H64634, AF0330951, B64815, YBHLE-COLI, EMEQUTR1, AF0647633, S53708, A69253, AF03541312 and S63281. (659 pages)

L14 ANSWER 22 OF 31 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-12901 BIOTECHDS
TITLE: New transmembrane **polypeptides** and nucleic acids encoding the polypeptides, useful in gene therapy, in chromosome identification, as chromosome markers, or in generating probes;

vector-mediated gene transfer and expression in host cell for recombinant protein production for use in disease diagnosis and gene therapy
AUTHOR: ASHKENAZI A J; BAKER K P; BOTSTEIN D; DESNOYERS L; EATON D L; FERRARA N; FONG S; GERBER H; GERRITSEN M E; GODDARD A; GODOWSKI P J; GRIMALDI J C; GURNEY A L; KLJAVIN I J; NAPIER M A; PAN J; PAONI N F; ROY M A; STEWART T A; TUMAS D;
WATANABE C K; WILLIAMS P M; WOOD W I; ZHANG Z
PATENT ASSIGNEE: GENENTECH INC
PATENT INFO: US 2002160384 31 Oct 2002
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PRIORITY INFO: WO 2001-21735 9 Jul 2001; WO 1997-20069 5 Nov 1997
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-288106 [28]
TI New transmembrane **polypeptides** and nucleic acids encoding the **polypeptides**, useful in gene therapy, in chromosome identification, as chromosome markers, or in generating probes;
vector-mediated gene transfer and expression in host cell for recombinant protein production for use in disease diagnosis and gene therapy
AN 2003-12901 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Isolated nucleic acid (I) comprising: (a) the full-length coding sequence of the DNA deposited under American Type Culture Collection (ATCC, accession numbers); (b) at least 80% sequence identity to a nucleotide sequence encoding a
polypeptide comprising a sequence selected from 149 fully defined amino acid sequences (P1-P149) all given in the specification; or (c) a sequence encoding any of **polypeptides** P1-P149 or the extracellular domain of P1-P149,
is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a vector comprising (I); (2) a host cell comprising the vector; (3) producing a PRO polypeptide by culturing the host cell for the expression of the PRO
polypeptide, and recovering the PRO **polypeptide** from the cell culture; (4) an isolated PRO **polypeptide** having at least 80% sequence identity to an amino acid sequence selected from P1-P149, or to the amino acid
sequence encoded by a nucleic acid molecule deposited with ATCC; (5) a chimeric molecule comprising a PRO **polypeptide** of (4) fused to a heterologous amino acid sequence; (6) an antibody which specifically binds to a PRO
polypeptide; (7) an isolated extracellular domain of PRO **polypeptide**; (8) an isolated PRO **polypeptide** lacking its associated signal **peptide**; (9) an isolated polypeptide having at least 80% amino
acid sequence identity to an extracellular domain of a PRO **polypeptide** or to a PRO **polypeptide** lacking its associated signal **peptide**; (10) detecting a PRO943, PRO183, PRO184, PRO185, PRO331, PRO113, PRO363,
PRO5723, PRO1387, PRO1114, PRO3301, PRO9940, PRO1181, PRO7170, PRO361, or PRO846 **polypeptide** in a sample suspected of containing the **polypeptide**; (11) linking a bioactive molecule to a cell expressing a PRO943, PRO183,
PRO184, PRO185, PRO331, PRO113, PRO363, PRO5723, PRO1387, PRO1114, PRO3301, PRO9940, PRO1181, PRO7170, PRO361, or PRO846 **polypeptide**; and (12) modulating at least one biological activity of a cell expressing a PRO943, PRO183, PRO184,
PRO185, PRO331, PRO113, PRO363, PRO5723, PRO1387, PRO1114, PRO3301, PRO9940, PRO1181, PRO7170, PRO361, or PRO846 **polypeptide**.
WIDER DISCLOSURE - Disclosed are: (A) agonists and antagonists of the polypeptides; and (B) identifying agonists and antagonists.
BIOTECHNOLOGY - Preferred Nucleic Acid: (I) comprises a sequence selected from 147 fully defined nucleotide sequences given in the specification. Preferred Host Cell: The host cell is a CHO cell, an E. coli cell or a yeast cell. Preferred
Chimeric Molecule: The heterologous amino acid sequence is an epitope tag sequence or an Fc region of an immunoglobulin. Preferred Antibody: The antibody is a monoclonal, humanized or an antibody fragment. Preferred Method: Detecting a PRO943

polypeptide in a sample containing the **polypeptide** comprises contacting the sample with a PRO183, PRO184 or PRO185 **polypeptide**, and determining the formation of a PRO943/PRO183, PRO184 or PRO185 **polypeptide**

conjugate in the sample indicating the presence of the PRO943 **polypeptide**. The sample comprises cells expressing PRO943 **polypeptide**. PRO183, PRO184 or PRO185 polypeptide is labeled with a detectable label or is

attached to a solid support. In detecting a PRO183, PRO184 or PRO185 polypeptide in a sample, the sample is contacted with PRO943, where formation of a PRO943/PRO183, PRO184 or PRO185 **polypeptide** conjugate indicates the

presence of PRO183, PRO184 or PRO185 **polypeptide** in the sample. The sample comprises cells expressing PRO183, PRO184 or PRO185 **polypeptide**. PRO943 is labeled with a detectable label or is attached to a solid support.

Detecting a PRO331 or PRO1133 **polypeptide** in a sample comprises contacting the sample with PRO1133 or PRO331, respectively, where formation of a PRO331/PRO1133 **polypeptide** conjugate indicates the presence of the

polypeptide in the sample. Detecting a PRO363 or PRO5723 **polypeptide** in a sample containing the **polypeptide** comprises contacting the sample with a PRO1387 polypeptide, and determining the formation of a

PRO363 or PRO5723/PRO1387 **polypeptide** conjugate in the sample indicating the presence of the PRO331 or PRO1133 **polypeptide**. Detecting a PRO1387 **polypeptide** in a sample containing the **polypeptide**

comprises contacting the sample with a PRO363 or PRO5723 **polypeptide**, and determining the formation of a PRO363 or PRO5723/PRO1387 **polypeptide** conjugate in the sample which indicates the presence of the PRO1387

polypeptide. Detecting a PRO1114 **polypeptide** in a sample containing the polypeptide comprises contacting the sample with a PRO3301 or PRO9940 **polypeptide**, and determining the formation of a PRO1114/PRO3301

or PRO9940 **polypeptide** conjugate in the sample which indicates the presence of the PRO1114 **polypeptide**. Detecting a PRO3301 or PRO9940 **polypeptide** in a sample containing the **polypeptide** comprises

contacting the sample with a PRO1114 **polypeptide**, and determining the formation of a PRO1114/PRO3301 or PRO9940 **polypeptide** conjugate in the sample which indicates the presence of the PRO3301 or PRO9940 **polypeptide**

. Detecting a PRO1181 **polypeptide** in a sample containing the **polypeptide** comprises contacting the sample with a PRO7170, PRO361 or PRO846 **polypeptide**, and determining the formation of a PRO1181/ PRO7170, PRO361 or

PRO846 **polypeptide** conjugate in the sample which indicates the presence of the PRO1181 **polypeptide**. Detecting a PRO7170, PRO361 or PRO846 **polypeptide** in a sample containing the **polypeptide** comprises

contacting the sample with a PRO1181 **polypeptide**, and determining the formation of a PRO1181/ PRO7170, PRO361 or PRO846 **polypeptide** conjugate in the sample which indicates the presence of the PRO7170, PRO361 or PRO846

polypeptide. The sample comprises cells suspected of expressing the polypeptide to be detected, and is contacted with a **polypeptide** labeled with a detectable label or which is attached to a solid support. Linking a

bioactive molecule to a cell expressing a PRO943 **polypeptide** comprises contacting the cell with a PRO183, PRO184 or PRO185 **polypeptide** that is bound to the bioactive molecule, and allowing the **polypeptides** to bind

to one another. Linking a bioactive molecule to a cell expressing a PRO183, PRO184 or PRO185 **polypeptide** comprises contacting the cell with a PRO943 **polypeptide** that is bound to the bioactive molecule, and allowing the

polypeptides to bind to one another. Linking a bioactive molecule to a cell expressing a PRO3301 or PRO1133 **polypeptide** comprises contacting the cell with a PRO1133 or PRO3301 **polypeptide**, respectively, bound to the

bioactive molecule, and allowing the **polypeptides** to bind to one another. Linking a bioactive molecule to a cell expressing a PRO1387 **polypeptide** comprises contacting the cell with a PRO363 or PRO5723 **polypeptide**

bound to the bioactive molecule, and allowing the **polypeptides** to bind to one another. Linking a bioactive molecule to a cell expressing a PRO363 or PRO5723 polypeptide comprises contacting the cell with a PRO1387

polypeptide bound to the bioactive molecule, and allowing the **polypeptides** to bind to one another. Linking a bioactive molecule to a cell expressing a PRO1144 polypeptide comprises contacting the cell with a PRO3301

or PRO9940 **polypeptide** bound to the bioactive molecule, and allowing the polypeptides to bind to one another. Linking a bioactive molecule to a cell expressing a PRO3301 or PRO9940 **polypeptide** comprises contacting the cell with a PRO1144 **polypeptide** bound to the bioactive molecule, and allowing the **polypeptides** to bind to one another. Linking a bioactive molecule to a cell expressing a PRO1181 **polypeptide** comprises contacting the cell with a PRO7170, PRO361 or PRO846 **polypeptide**, bound to the bioactive molecule, and allowing the **polypeptides** to bind to one another. Linking a bioactive molecule to a cell expressing a PRO7170, PRO361 or PRO846 **polypeptide** comprises contacting the cell with a PRO1181 **polypeptide** bound to the bioactive molecule, and allowing the **polypeptides** to bind to one another. The bioactive molecule is a toxin, a radiolabel or an antibody. The bioactive molecule may cause the death of the cell. Modulating at least one biological activity of a cell expressing PRO943 **polypeptide** comprises contacting the cell with a PRO183, PRO184 or PRO185 **polypeptide**, or an anti-PRO943 antibody, where the **polypeptide** or the antibody binds to PRO943 **polypeptide** to modulate at least one biological activity of the cell, where the cell is killed. Modulating at least one biological activity of a cell expressing a PRO183, PRO184 or PRO185 polypeptide comprises contacting the cell with a PRO943 **polypeptide**, or an anti-PRO183, anti-PRO184 or anti-PRO185 antibody, where the **polypeptide** or antibody binds to PRO183, PRO184 or PRO185 **polypeptide** to modulate at least one biological activity of the cell, where the cell is killed. Modulating at least one biological activity of a cell expressing a PRO1133 or PRO331 **polypeptide** comprises contacting the cell with a PRO331 or PRO1133 **polypeptide**, or an anti-PRO331 or anti-PRO1133 antibody, where the **polypeptide** or antibody binds to PRO1133 or PRO331 **polypeptide** to modulate at least one biological activity of the cell, where the cell is killed. Modulating at least one biological activity of a cell expressing PRO1387 polypeptide comprises contacting the cell with a PRO363 or PRO5723 **polypeptide**, or an anti-PRO1387 antibody, where the **polypeptide** or the antibody binds to PRO1387 **polypeptide** to modulate at least one biological activity of the cell, where the cell is killed. Modulating at least one biological activity of a cell expressing PRO363 or PRO5723 **polypeptide** comprises contacting the cell with a PRO1387 **polypeptide**, or an anti-PRO363 or anti-PRO5723 antibody, where the **polypeptide** or the antibody binds to PRO363 or PRO5723 **polypeptide** to modulate at least one biological activity of the cell, where the cell is killed. Modulating at least one biological activity of a cell expressing PRO1114 **polypeptide** comprises contacting the cell with a PRO3301 or PRO9940 **polypeptide**, or an anti-PRO1114 antibody, where the **polypeptide** or the antibody binds to PRO1114 polypeptide to modulate at least one biological activity of the cell, where the cell is killed. Modulating at least one biological activity of a cell expressing PRO3301 or PRO9940 **polypeptide** comprises contacting the cell with a PRO1114 **polypeptide**, or an anti-PRO3301 or anti-PRO9940 antibody, where the **polypeptide** or the antibody binds to PRO3301 or PRO9940 **polypeptide** to modulate at least one biological activity of the cell, where the cell is killed. Modulating at least one biological activity of a cell expressing PRO1181 **polypeptide** comprises contacting the cell with a PRO7170, PRO361 or PRO846 **polypeptide**, or an anti-PRO1181 antibody, where the **polypeptide** or the antibody binds to PRO1181 **polypeptide** to modulate at least one biological activity of the cell, where the cell is killed. Modulating at least one biological activity of a cell expressing PRO7170, PRO361 or PRO846 **polypeptide** comprises contacting the cell with a PRO1181 **polypeptide**, or an anti-PRO7170, anti-PRO361 or anti-PRO846 antibody, where the **polypeptide** or the antibody binds to PRO7170, PRO361 or PRO846 **polypeptide** to modulate at least one biological activity of the cell, where the cell is killed.

ACTIVITY - None given.

MECHANISM OF ACTION - Gene therapy.

USE - Nucleic acids which encode PRO can be used to generate either transgenic animals or knock-out animals which may be used in the development and screening of therapeutically useful reagents. The nucleic acids may also be used in gene

therapy, in chromosome identification, as chromosome markers, or in generating probes. The PRO **polypeptides** are useful as molecular markers for protein electrophoresis, and the isolated nucleic acids may be used for recombinantly expressing those markers. The PRO **polypeptides** and nucleic acids may also be used in tissue typing. Anti-PRO antibodies are useful in diagnostic assays for PRO, and in affinity purification of PRO from recombinant ell culture or natural sources.

ADMINISTRATION - Dosage is 10 ng/kg-100 mg/kg, preferably 1 mug/kg/day-10 mg/kg/day. Administration can be through injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial or intralesional routes, topical, or by **sustained release** systems.

EXAMPLE - Yeast transformation was performed with limiting amounts of transforming DNA to reduce the number of multiple transformed yeast cells. Instead of plasmid isolation form the yeast followed by transformation of E. coli, PCR was performed on single yeast colonies using bipartite primers to amplify the insert and a small portion of the invertase gene and to add on universal sequencing primer sites. An invertase library was transformed into yeast and positives were selected on **sucrose** plates. Positive clones were re-tested and PCR products were sequenced. The sequence of one clone, PRO281, was determined to contain a signal **peptide** coding sequence. Oligonucleotide primers and probes were designed using the nucleotide sequence of PRO281. A full length plasmid library of cDNAs from human umbilical vein endothelium tissue was tittered and about 100000 cfu were plated in 192 pools of 500 cfu/pool into 96-2311 round bottom plates, and were incubated overnight with shaking. PCR was performed on individual cultures and agarose gel electrophoresis was performed where positive wells were identified by visualization of a band of expected size. Individual positive clones were obtained by colony lift followed by hybridization with 32-labeled oligonucleotide. Clones were characterized by PCR, restriction digest and southern blot. A full length clone was identified and contained a single open reading frame with an apparent translational initiation site at nucleotide positions 80-82, and a stop signal at nucleotide positions 1115-1117. The predicted **polypeptide** precursor is 345 amino acids long. (650 pages)

L14 ANSWER 23 OF 31 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-11032 BIOTECHDS
TITLE: Novel isolated PRO **polypeptides** e.g., PRO826, PRO1068, PRO1184, PRO1346 and PRO1375, which stimulate proliferation of stimulated T-lymphocytes are therapeutically useful for enhancing immune response and in cancer treatments;
vector expression in CHO cell and Escherichia coli for recombinant protein production and disease therapy and gene therapy
AUTHOR: ASHKENAZI A J; BAKER K P; BOTSTEIN D; DESNOYERS L; EATON D L; FERRARA N; FONG S; GERBER H; GERRITSEN M E; GODDARD A; GODOWSKI P J; GRIMALDI J C; GURNEY A L; KLJAVIN I J; NAPIER M A; PAN J; PAONI N F; ROY M A; STEWART T A; TUMAS D;
WATANABE C K; WILLIAMS P M; WOOD W I; ZHANG Z
PATENT ASSIGNEE: GENENTECH INC
PATENT INFO: US 2002132252 19 Sep 2002
APPLICATION INFO: US 2001-990442 14 Nov 2001
PRIORITY INFO: WO 2001-21735 9 Jul 2001; WO 1997-20069 5 Nov 1997
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-247083 [24]
TI Novel isolated PRO **polypeptides** e.g., PRO826, PRO1068, PRO1184, PRO1346 and PRO1375, which stimulate proliferation of stimulated T-lymphocytes are therapeutically useful for enhancing immune response and in cancer treatments;
vector expression in CHO cell and Escherichia coli for recombinant protein production and disease therapy and gene therapy
AN 2003-11032 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An isolated PRO **polypeptide** (I) having at least 80% sequence identity to (i) an amino acid sequence chosen from any one of fully defined 147 polypeptide sequences (PS) as given in the specification, or (ii) to PS lacking its associated signal **peptide**, or an isolated extracellular domain of PRO **polypeptide** with or without its associated signal **peptide**, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid (II) having at least 80% identity to a nucleotide sequence that encodes PS; (2) an isolated nucleic acid comprising the full-length coding sequence of DNA chosen from any of 133 DNAs deposited in ATCC as given in the specification; (3) a vector (III) comprising (II); (4) a host cell (IV) comprising (III); (5) preparation of (I); (6) an isolated PRO **polypeptide** having at least 80% sequence identity to the amino acid sequence encoded by nucleic acid molecule deposited under any of ATCC accession number as described above; (7) a chimeric molecule (V) comprising (I) fused to a heterologous amino acid sequence; (8) an antibody (VI) which specifically binds to (I); (9) an isolated nucleic acid molecule (VII) comprising 80% sequence identity to (a) a nucleic acid comprising a sequence chosen from any one of 147 fully defined PRO polynucleotide sequences (NS) as given in the specification, or (b) the full-length coding sequence of NS; (10) an isolated extracellular domain (VIII) of the PRO **polypeptide**; (11) an isolated PRO **polypeptide** (IX) lacking its associated signal **peptide**; (12) an isolated **polypeptide** having at least 80% amino acid sequence identity (VIII) or (IX); and (13) isolated nucleic acid having 80% nucleic acid sequence identity to a nucleotide sequence encoding PS, lacking its associated signal **peptide**; or a nucleotide sequence encoding (VIII) with or without its associated signal **peptide**.

WIDER DISCLOSURE - The following are also disclosed: (1) nucleic acids complementary to above mentioned nucleic acids; (2) nucleic acids having at least 10 nucleotides and produced by hybridizing test DNA molecule under stringent conditions with DNA molecule encoding PRO **polypeptide**, or its complement; (3) fragments of PRO **polypeptide** coding sequence; (4) a composition comprising PRO polypeptide, its agonists or antagonists, anti-PRO **polypeptide** useful in treatment of conditions responsive to PRO **polypeptide**, its agonists or antagonists or anti-PRO antibodies; (5) agonists and antagonists of PRO **polypeptides**; (6) variants of (I); (7) covalent modifications of (I); (8) detecting a PRO **polypeptide**; (9) linking a bioactive molecule to a cell expressing a PRO **polypeptide**; (10) modulating at least one biological activity of a cell expressing a PRO **polypeptide**;

BIOTECHNOLOGY - Preparation: (I) is prepared by standard recombinant techniques (claimed). Preferred Polynucleotide: (II) comprises a full-length coding sequence of a sequence chosen from any one of 147 fully defined PRO polynucleotide sequences as given in the specification. Preferred Vector: (III) comprises (II) operably linked to the control sequences recognized by a host cell transformed with the vector. Preferred Host Cell: (IV) is a Chinese hamster ovary cell or an Escherichia coli cell. Preferred Molecule: (V) comprises (I) fused to an epitope tag sequence or to an immunoglobulin Fc region. Preferred Methods: (I) is useful for detecting the PRO **polypeptides** in sample e.g., PRO943 **polypeptide** is detected in a sample suspected of containing a PRO943 polypeptide, by contacting the sample with a PRO183, PRO184 or PRO185 **polypeptide** and determining the formation of PRO943/PRO183, PRO184, or PRO185 **polypeptide** conjugate; and the presence of PRO183, PRO184 or PRO185 polypeptide in a sample is detected by contacting PRO943 **polypeptide** and determining the formation of the conjugate as described above.

Similarly, the **polypeptides** PRO331; PRO363 or PRO5723; PRO1114; PRO1181 are detected by contacting sample with PRO1133; PRO1387; PRO3301 or PRO9940; PRO7170, PRO361 or PRO846, **polypeptides**, respectively and determining formation of conjugate. And for detecting PRO1133; PRO1387; PRO3301 or PRO9940; PRO7170, PRO361 or PRO846, **polypeptides**, the sample is contacted with PRO331; PRO363 or PRO5723; PRO1114; PRO1181 **polypeptides**, respectively, and formation of conjugate is determined. The sample comprises cells suspected of expressing the PRO **polypeptide**. PRO **polypeptides** which are contacted with the ample are labeled with a detectable label or a solid support.

The PRO **polypeptides** are also useful for linking a bioactive molecule to a cell expressing a PRO **polypeptide**, e.g. PRO183, PRO184 or PRO185; PRO1133; PRO363 or PRO5723; PRO3301 or PRO9940; PRO7170, PRO361 or PRO846,

polypeptides are useful for linking a bioactive molecule to a cell expressing PRO943; PRO331; PRO1387; PRO1114; PRO1181 **polypeptides**, respectively, and the polypeptides PRO943; PRO331; PRO1387; PRO1114; PRO1181 are

useful for linking a bioactive molecule to a cell expressing PRO183, PRO184 or PRO185; PRO1133; PRO363 or PRO5723; PRO3301 or PRO9940; PRO7170, PRO361 or PRO846, **polypeptides**, respectively. The bioactive molecule is a toxin,

radiolabel or antibody, which causes the death of the cell. The PRO polypeptides are useful for modulating a biological activity of a cell expressing the PRO **polypeptides**, e.g. the biological activity of a cell expressing

PRO183, PRO184 or PRO185 is modulated by contacting the cell with PRO943 polypeptide or anti-PRO183, anti-PRO184 or anti-PRO185 antibody, such that the cell is killed. Similarly, other PRO **polypeptides** are useful for

modulating the biological activity of the cell expressing the counterpart polypeptides as described above.

ACTIVITY - Vulnerary; Antitumor; Antiarthritic; Cytostatic; Antiinflammatory; Cardiant; Nephrotropic; Immunomodulatory; Ophthalmological. No supporting data provided.

MECHANISM OF ACTION - PRO antagonist; Gene therapy; Stimulates hypertrophy of neonatal heart; Stimulates adrenal cortical capillary endothelial growth; Inhibits vascular endothelial growth factor (VEGF) stimulated proliferation of

endothelial cells; Stimulates proliferation of stimulated T-lymphocytes; Enhances survival of rod photoreceptor cells or neuronal cells; Induces proliferation of mammalian kidney mesangial cells; Induces proliferation and/or redifferentiation

of chondrocytes in culture. The ability of the PRO **polypeptides** PRO1310, PRO844, PRO1312, PRO1192 and PRO1387 to induce the proliferation and/or redifferentiation of chondrocyte in culture was tested in a chondrocyte proliferation

assay. Porcine chondrocytes were isolated by overnight collagenase digestion of articular cartilage of the metacarpophalngeal joint of 4-6 month old female pigs. The isolated cells were then seeded at 25000 cells/cm2 in Ham F-12. On day 12,

the cells were seeded in 96-well plates at 5000 cells/well in 100mul of the media without serum and 100 mul of either serum-free medium, staurosporin, or the test PRO **polypeptide**. After 5 days, at 37degreesC, 2 mul of Alamar blue was

added to each well and the plates were incubated for an additional 3 hours at 37degreesC. The fluorescence was then measured in each well. The PRO **polypeptide** -treated sample provided a positive result, i.e., a fluorescence more

likely that of the positive control than the negative control.

USE - The PRO **polypeptides** are useful in detecting PRO **polypeptides** in a sample, in linking a bioactive molecule to a cell expressing a PRO **polypeptide**, and in modulating at least one biological activity of a

cell expressing a PRO **polypeptide**. The PRO **polypeptides** are useful as molecular weight markers for protein electrophoresis. (I) is also useful for screening compounds to identify those that mimic the PRO **polypeptide**

(agonists) or prevent the effect of the PRO **polypeptide** (antagonists). (II) is useful as hybridization probes. (I) is also useful for screening compounds to identify those that mimic the PRO **polypeptide** (agonists) or

prevent the effect of the PRO **polypeptide** (antagonists). The PRO **polypeptides** are also useful as therapeutic agents. PRO1312 stimulates hypertrophy of neonatal heart and is thus useful for treating cardiac insufficiency

disorders. PRO1154 and PRO1186 stimulate adrenal cortical capillary endothelial growth, and are thus useful for treating conditions or disorders where angiogenesis would be beneficial, e.g. wound healing and antagonist of this

polypeptide (e.g. antibodies against the **polypeptide**) are useful for treating cancerous tumors. PRO812 inhibits vascular endothelial growth factor (VEGF) stimulated proliferation of endothelial cells and is thus useful for

inhibiting endothelial cell growth in mammals which would be beneficial in inhibiting tumor growth. PRO826, PRO1068, PRO1184, PRO1346 and PRO1375 stimulate proliferation of stimulated T-lymphocytes and are therapeutically useful for enhancing

immune response. PRO828, PRO826, PRO1068 or PRO1132 enhance survival of retinal neurons cells and therefore are useful for treating retinal disorders of injuries, e.g. sight loss in mammals due treating retinitis pigmentosum, AMD. PRO536,

PRO943, PRO828, PRO826, PRO1068 or PRO1132 enhance survival/proliferation of rod photoreceptor cells and therefore are useful for treating retinal disorders of injuries, e.g. sight loss in mammals due to retinitis pigmentosum, AMD. PRO535,

PRO826, PRO819, PRO1126, PRO1360 and PRO1387 induce c-fos in endothelial cells, and are thus useful for treating conditions or disorders where angiogenesis would be beneficial, e.g. wound healing and antagonist of this **polypeptide**

(e.g. antibodies against the **polypeptide**) are useful for treating cancerous tumors. PRO819, PRO813 and PRO11066 induce proliferation of mammalian kidney mesangial cells, and therefore are useful for treating kidney disorders

associated with decreased mesangial cell function such as Berger disease or other nephropathies associated with dermatitis, herpetiformis or Crohn's disease. PRO1310, PRO844, PRO1312, PRO1192 and PRO1387 induce the proliferation and/or

redifferentiation of chondrocytes in culture and are thus useful for treating sports injuries, and arthritis.

ADMINISTRATION - Administered by injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial or intralesional route, topical route or by **sustained release** systems.

The PRO **polypeptide** is administered at a dose of 10 ng/kg-100 mg/kg of mammal body weight or more/day, preferably 1 mug/kg/day-10 mg/kg/day.

EXAMPLE - Isolation of cDNA clones encoding human PRO281 was carried out as follows. In order to obtain a cDNA clone encoding PRO281, methods described in Klein et al., Proc. Natl. Acad. Sci. USA 93:7108-7113 (1996) were employed with the

following modifications. Yeast transformation was performed with limiting amounts of transforming DNA in order to reduce the number of multiple transformed yeast cells. Instead of plasmid isolation from the yeast followed by transformation of

Escherichia coli. Polymerase chain reaction (PCR) analysis was performed on single yeast colonies. PCR primers employed were bipartite in order to amplify the insert and a small portion of the invertase gene (allowing to determine that the

insert was in frame with invertase) and to add on universal sequencing primer sites. An invertase library was transformed into yeast and positives were selected on **sucrose** plates. Positive clones were re-tested and PCR products were

sequenced. The sequence of one clone, PRO281, was determined to contain a signal **peptide** coding sequence. Oligonucleotide primers and probes were designed using the nucleotide sequence of PRO281. A full length plasmid library of

cDNAs from human umbilical vein endothelium tissue was titered and 100000 cfu (colony forming units) were plated in 192 pools of 500 cfu/pool into 96-well round bottom plates. The plates were sealed and pools were grown overnight at 37degreesC

with shaking. PCR was performed on the individual cultures using primers. Agarose gel electrophoresis was performed and positive wells were identified. Individual positive clones were obtained by colony lift followed by hybridization with

32P-labeled oligonucleotide. These clones were characterized by PCR, restriction digest, and southern blot analyses. A full length clone was identified that contained a single open reading frame with an apparent translational initiation site

at nucleotide positions 80-82 and a stop signal at nucleotide positions 1115-1117. The predicted **polypeptide** precursor is 345 amino acids (S1) long, has a calculated molecular weight of 37205 Da and an estimated pI of 10.15. Analysis

of the full-length PRO281 sequence of (S1) showed the presence of the following: a signal **peptide** from amino acid 1-14, multiple transmembrane domains from amino acids 83-105, 126-146, 158-177, 197-216, 218-238, 245-265, 271-290, and

an amino acid sequence block having homology to G-protein coupled receptor proteins from amino acid 115-155. Clone UNQ244 (DNA16422-1209) was deposited with ATCC on Jun 2, 1998 and is assigned ATCC deposit no.209929. An analysis of the Dayhoff

database (version 35,45 SwissProt 25), using a WU-BLAST-2 sequence alignment analysis of the full-length sequence of PRO281, evidenced significant homology between the PRO281 amino acid sequence and the following Dayhoff sequences: H64634,

AF0330951, B64815, YBHLE-COLI, EMEQUTR1, AF0647633, S53708, A69253, AF03541312 and S63281. (648 pages)

L14 ANSWER 24 OF 31 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-14203 BIOTECHDS
TITLE: Novel isolated PRO **polypeptides** e.g., PRO826, PRO1068, PRO1184, PRO1346 and PRO1375, which stimulate proliferation of stimulated T-lymphocytes and are therapeutically useful for enhancing immune responses;
vector-mediated gene transfer and expression in host cell for recombinant protein production, drug screening and gene therapy
AUTHOR: ASHKENAZI A J; BAKER K P; BOTSTEIN D; DESNOYERS L; EATON D L; FERRARA N; FONG S; GERBER H; GERRITSEN M E; GODDARD A; GODOWSKI P J; GRIMALDI J C; GURNEY A L; KLJAVIN I J; NAPIER M A; PAN J; PAONI N F; ROY M A; STEWART T A; TUMAS D;
WATANABE C K; WILLIAMS P M; WOOD W I; ZHANG Z
PATENT ASSIGNEE: GENENTECH INC
PATENT INFO: US 2002127576 12 Sep 2002
APPLICATION INFO: US 2001-991073 14 Nov 2001
PRIORITY INFO: WO 2001-21735 9 Jul 2001; WO 1997-20069 5 Nov 1997
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-340824 [32]
TI Novel isolated PRO **polypeptides** e.g., PRO826, PRO1068, PRO1184, PRO1346 and PRO1375, which stimulate proliferation of stimulated T-lymphocytes and are therapeutically useful for enhancing immune responses;
vector-mediated gene transfer and expression in host cell for recombinant protein production, drug screening and gene therapy
AN 2003-14203 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An isolated PRO **polypeptide** (I) having at least 80% sequence identity to: (i) an amino acid sequence chosen from any one of fully defined 147 polypeptide sequences (PS) as given in the specification; or (ii) to PS
lacking its associated signal **peptide**, or an isolated extracellular domain of PRO **polypeptide** with or without its associated signal **peptide**, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) an isolated nucleic acid (II) having at least 80% identity to a nucleotide sequence that encodes PS; (2) an isolated nucleic acid comprising the full-length coding
sequence of DNA chosen from any of 141 DNAs deposited in ATCC as given in the specification; (3) a vector (III) comprising (II); (4) a host cell (IV) comprising (III); (5) preparation of (I); (6) an isolated PRO **polypeptide** having at
least 80% sequence identity to the amino acid sequence encoded by nucleic acid molecule deposited under any of ATCC accession number as described above; (7) a chimeric molecule (V) comprising (I) fused to a heterologous amino acid sequence;
(8) an antibody (VI) which specifically binds to (I); (9) an isolated nucleic acid molecule (VII) comprising 80% sequence identity to: (a) a nucleic acid comprising a sequence chosen from any one of 147 fully defined PRO polynucleotide
sequences (NS) as given in the specification; or (b) the full-length coding sequence of NS; (10) an isolated extracellular domain (VIII) of the PRO **polypeptide** ; (11) an isolated PRO **polypeptide** (IX) lacking its associated
signal **peptide**; (12) an isolated **polypeptide** having at least 80% amino acid sequence identity (VIII) or (IX); and (13) isolated nucleic acid having 80% nucleic acid sequence identity to a nucleotide sequence encoding PS,
lacking its associated signal **peptide**, or a nucleotide sequence encoding (VIII) with or without its associated signal **peptide**.
WIDER DISCLOSURE - The following are disclosed: (1) nucleic acids complementary to above mentioned nucleic acids; (2) nucleic acids having at least 10 nucleotides and produced by hybridizing test DNA molecule under stringent conditions
with DNA molecule encoding PRO **polypeptide**, or its complement; (3) fragments of PRO **polypeptide** coding sequence; (4) a composition comprising PRO **polypeptide**, its agonists or antagonists, anti-PRO
polypeptide useful in treatment of conditions responsive to PRO **polypeptide**, its agonists or antagonists or anti-PRO antibodies; (5) agonists and antagonists of PRO **polypeptides**; (6) variants of (I); and (7)

covalent modifications of (I).

BIOTECHNOLOGY - Preparation: (I) is prepared by standard recombinant techniques (claimed). Preferred Polynucleotide: (II) comprises a full-length coding sequence of a sequence chosen from any one of 147 fully defined PRO polynucleotide sequences as given in the specification. Preferred Vector: (III) comprises (II) operably linked to the control sequences recognized by a host cell transformed with the vector. Preferred Host Cell: (IV) is a Chinese hamster ovary cell or an Escherichia coli cell. Preferred Molecule: (V) comprises (I) fused to an epitope tag sequence or to an immunoglobulin Fc region.

ACTIVITY - Vulnerary; Antitumor; Antiarthritic.

MECHANISM OF ACTION - PRO antagonist; Gene therapy; Stimulates hypertrophy of neonatal heart; Stimulates adrenal cortical capillary endothelial growth; Inhibits vascular endothelial growth factor (VEGF) stimulated proliferation of endothelial cells; Stimulates proliferation of stimulated T-lymphocytes; Enhances survival of rod photoreceptor cells or neuronal cells; Induces proliferation of mammalian kidney mesangial cells; Induces proliferation and/or redifferentiation of chondrocytes in culture. The ability of the PRO **polypeptides** PRO1310, PRO844, PRO1312, PRO1192 and PRO1387 to induce the proliferation and/or redifferentiation of chondrocyte in culture was tested in a chondrocyte proliferation assay. Porcine chondrocytes were isolated by overnight collagenase digestion of articular cartilage of the metacarpophalngeal joint of 4-6 month old female pigs. The isolated cells were then seeded at 25000 cells/cm2 in Ham F-12. On day 12, the cells were seeded in 96-well plates at 5000 cells/well in 100 microl of the media without serum and 100 microl of either serum-free medium, staurosporin, or the test PRO **polypeptide**. After 5 days, at 37degreesC, 2 microl of Alamar blue was added to each well and the plates were incubated for an additional 3 hours at 37 degreesC. The fluorescence was then measured in each well. The PRO **polypeptide**-treated sample provided a positive result, i.e. a fluorescence more likely that of the positive control than the negative control.

USE - (I) is useful for detecting the PRO **polypeptides** in sample e.g., PRO943 **polypeptide** is detected in a sample suspected of containing a PRO943 polypeptide, by contacting the sample with a PRO183, PRO184 or PRO185 **polypeptide** and determining the formation of PRO943/PRO183, PRO184, or PRO185 **polypeptide** conjugate; and the presence of PRO183, PRO184 or PRO185 polypeptide in a sample is detected by contacting PRO943 **polypeptide** and determining the formation of the conjugate as described above. Similarly, the **polypeptides** PRO331; PRO363 or PRO5723; PRO1114; PRO1181 are detected by contacting sample with PRO1133; PRO1387; PRO3301 or PRO9940; PRO7170, PRO361 or PRO846, **polypeptides**, respectively and determining formation of conjugate. And for detecting PRO1133; PRO1387; PRO3301 or PRO9940; PRO7170, PRO361 or PRO846, **polypeptides**, the sample is contacted with PRO331; PRO363 or PRO5723; PRO1114; PRO1181 **polypeptides**, respectively, and formation of conjugate is determined. The sample comprises cells suspected of expressing the PRO **polypeptide**. PRO **polypeptides** which are contacted with the ample are labeled with a detectable label or a solid support. The PRO **polypeptides** are also useful for linking a bioactive molecule to a cell expressing a PRO **polypeptide**, e.g. PRO183, PRO184 or PRO185; PRO1133; PRO363 or PRO5723; PRO3301 or PRO9940; PRO7170, PRO361 or PRO846, **polypeptides** are useful for linking a bioactive molecule to a cell expressing PRO943; PRO331; PRO1387; PRO1114; PRO1181 **polypeptides**, respectively, and the **polypeptides** PRO943; PRO331; PRO1387; PRO1114; PRO1181 are useful for linking a bioactive molecule to a cell expressing PRO183, PRO184 or PRO185; PRO1133; PRO363 or PRO5723; PRO3301 or PRO9940; PRO7170, PRO361 or PRO846, **polypeptides**, respectively. The bioactive molecule is a toxin, radiolabel or antibody, which causes the death of the cell. The PRO **polypeptides** are useful for modulating a biological activity of a cell expressing the PRO **polypeptides**, e.g. the biological activity of a cell expressing PRO183, PRO184 or PRO185 is modulated by contacting the cell with PRO943 **polypeptide** or anti-PRO183, anti-PRO184 or anti-PRO185 antibody, such that the

cell is killed. Similarly, other PRO **polypeptides** are useful for modulating the biological activity of the cell expressing the counterpart **polypeptides** as described above (all claimed).

ADMINISTRATION - Administered by injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial or intralesional route, topical route or by **sustained release** systems.

The PRO **polypeptide** is administered at a dose of 10 ng/kg-100 mg/kg of mammal body weight or more/day, preferably 1 microg/kg/day-10 mg/kg/day.

EXAMPLE - Isolation of cDNA clones encoding human PRO281 was carried out as follows. In order to obtain a cDNA clone encoding PRO281, methods described in Klein et al., Proc. Natl. Acad. Sci. USA 93:7108-7113 (1996) were employed with the following modifications. Yeast transformation was performed with limiting amounts of transforming DNA in order to reduce the number of multiple transformed yeast cells. Instead of plasmid isolation from the yeast followed by transformation of

Escherichia coli. Polymerase chain reaction (PCR) analysis was performed on single yeast colonies. PCR primers employed were bipartite in order to amplify the insert and a small portion of the invertase gene (allowing to determine that the insert was in frame with invertase) and to add on universal sequencing primer sites. An invertase library was transformed into yeast and positives were selected on **sucrose** plates. Positive clones were re-tested and PCR products were sequenced. The sequence of one clone, PRO281, was determined to contain a signal **peptide** coding sequence. Oligonucleotide primers and probes were designed using the nucleotide sequence of PRO281. A full length plasmid library of cDNAs from human umbilical vein endothelium tissue was titered and 100000 cfu (colony forming units) were plated in 192 pools of 500 cfu/pool into 96-well round bottom plates. The plates were sealed and pools were grown overnight at 37 degreesC with shaking. PCR was performed on the individual cultures using primers. Agarose gel electrophoresis was performed and positive wells were identified. Individual positive clones were obtained by colony lift followed by hybridization with 32P-labeled oligonucleotide. These clones were characterized by PCR, restriction digest, and southern blot analyses. A full length clone was identified that contained a single open reading frame with an apparent translational initiation site at nucleotide positions 80-82 and a stop signal at nucleotide positions 1115-1117. The predicted **polypeptide** precursor is 345 amino acids (S1) long, has a calculated molecular weight of 37205 Da and an estimated pI of 10.15.

Analysis of the full-length PRO281 sequence of (S1) showed the presence of the following: a signal **peptide** from amino acid 1-14, multiple transmembrane domains from amino acids 83-105, 126-146, 158-177, 197-216, 218-238, 245-265, 271-290, and an amino acid sequence block having homology to G-protein coupled receptor proteins from amino acid 115-155. Clone UNQ244 (DNA16422-1209) was deposited with ATCC on Jun 2, 1998 and is assigned ATCC deposit no.209929. An analysis of the Dayhoff database (version 35,45 SwissProt 25), using a WU-BLAST-2 sequence alignment analysis of the full-length sequence of PRO281, evidenced significant homology between the PRO281 amino acid sequence and the following Dayhoff sequences: H646344, AF0330951, B64815, YBHLE-COLI, EMEQUTR1, AF0647633, S53708, A69253, AF03541312 and S63281.(661 pages)

L14 ANSWER 25 OF 31 PASCAL COPYRIGHT 2005 INIST-CNRS. ALL RIGHTS RESERVED. on STN DUPLICATE 2

ACCESSION NUMBER: 2001-0136562 PASCAL

TITLE (IN ENGLISH): GnRH antagonists : A new generation of long acting analogues incorporating p-ureido-phenylalanines at positions 5 and 6

AUTHOR: GUANGCHENG JIANG; STALEWSKI Jacek; GALYEAN Robert; DYKERT John; SCHTEINGART Claudio; BROQUA Pierre; AEBI Audrey; AUBERT Michel L.; SEMPLE Graeme; ROBSON Peter; AKINSANYA Karen; HAIGH Robert; RIVIERE Pierre; TROJNAR Jerzy; JUNIEN Jean Louis; RIVIER Jean E.

CORPORATE SOURCE: Ferring Research Institute Inc., San Diego, California 92121, United States; Department of Pediatrics, University of Geneva, School of Medicine, 1211 Geneva, Switzerland; Ferring Research Institute Ltd., Southampton SO16

7NP, United Kingdom; Ferring SAS, 216 Boulevard Saint Germain, 75007 Paris, France; The Salk Institute, La Jolla, California 92037, United States

SOURCE: Journal of medicinal chemistry : (Print), (2001), 44(3), 453-467, 44 refs.
ISSN: 0022-2623 CODEN: JMCMAR
DOCUMENT TYPE: Journal
BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: United States
LANGUAGE: English
AVAILABILITY: INIST-9165, 354000094173390150

TIEN GnRH antagonists : A new generation of long acting analogues incorporating p-ureido-phenylalanines at positions 5 and 6

AN 2001-0136562 PASCAL

AB A series of antagonists of gonadotropin-releasing hormone (GnRH) of the general formula Ac-D2Nal-D4Cpa-D3Pal-Ser-4Aph/4Amf(P)-D4Aph/D4Amf(Q)-Leu-ILys-Pro-DAla-NH.sub.2 was synthesized, characterized, and screened for duration of inhibition of luteinizing hormone release in a castrated male rat assay. Selected analogues were tested in a reporter gene assay (IC.sub.5.sub.0 and pA.sub.2) and an in vitro histamine release assay. P and Q contain urea/carbamoyl functionalities designed to increase potential intra- and intermolecular hydrogen bonding opportunities for structural stabilization and **peptide**/receptor interactions, respectively. These substitutions resulted in analogues with increased hydrophilicity and a lesser propensity to form gels in aqueous solution than azaline B [Ac-D2Nal-D4Cpa-D3Pal-Ser-4Aph(Atz)-D4Aph(Atz)-Leu-ILys-Pro-DAla-NH.sub.2 with Atz = 3'-amino-1H-1',2',4'.-triazol-5'-yl, 5], and in some cases they resulted in a significant increase in duration of action after subcutaneous (sc) administration. Ac-D2Nal-D4Cpa-D3Pal-Ser-4Aph(L-hydroorotyl)-D4Aph(carbamoyl)-Leu-ILys-Pro-DAla-NH.sub.2 (acetate salt is FE200486) (31) and eight other congeners (20, 35, 37, 39, 41, 45-47) were identified that exhibited significantly longer duration of action than acyline [Ac-D2Nal-D4Cpa-D3Pal-Ser-4Aph-(Ac)-D4Aph(Ac)-Leu-ILys-Pro-DAla-NH.sub.2] (6) when administered subcutaneously in castrated male rats at a dose of 50 μg in 100 μL of phosphate buffer. No correlation was found between retention times on a C.sub.1.sub.8 reverse phase column using a triethylammonium phosphate buffer at pH 7.0 (a measure of hydrophilicity) or affinity in an in vitro human GnRH report gene assay (pA.sub.2) and duration of action. FE200486 was selected for preclinical studies, and some of its properties were compared to those of other clinical candidates. In the intact rat, ganirelix, abarelix, azaline B, and FE200486 inhibited plasma testosterone for 1, 1, 14, and 57 days, respectively, at 2 mg/kg sc in 5% **mannitol** (injection volume = 20 μL). Based on the information that 31, 33, 35 and 37 were significantly shorter acting than acyline or azaline B after intravenous administration (100 μg/rat), we surmised that the very long duration of action of the related FE200486 (for example) was likely due to unique physicochemical properties such as solubility in aqueous milieu, comparatively low propensity to form gels, and ability to diffuse at high concentrations in a manner similar to that described for **slow release** formulations of **peptides**. Indeed, in rats injected sc with FE200486 (2 mg/kg), plasmatic concentrations of FE200486 remained above 5 ng/mL until day 41, and the time after which they dropped below 3 ng/mL and plasma LH levels started rising until full recovery was reached at day 84 with levels of FE200486 hovering around 1 ng/mL. Additionally, FE200486 was less potent at releasing histamine from isolated rat mast cells than any of the GnRH antagonists presently described in preclinical reports.

L14 ANSWER 26 OF 31 MEDLINE on STN

ACCESSION NUMBER: 1998167329 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9507911
TITLE: Delayed gastric emptying occurs following acarbose administration and is a further mechanism for its anti-hyperglycaemic effect.
AUTHOR: Ranganath L; Norris F; Morgan L; Wright J; Marks V
CORPORATE SOURCE: Epsom General Hospital, Surrey, UK.
SOURCE: Diabetic medicine : a journal of the British Diabetic Association, (1998 Feb) 15 (2) 120-4.
Journal code: 8500858. ISSN: 0742-3071.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199804
ENTRY DATE: Entered STN: 19980422
Last Updated on STN: 20000303
Entered Medline: 19980414

TI Delayed gastric emptying occurs following acarbose administration and is a further mechanism for its anti-hyperglycaemic effect.
AB The therapeutic effect of acarbose is generally attributed to inhibition of amylase and brush border glucosidases and consequent impaired digestion and absorption of carbohydrates. We have investigated the possibility that acarbose may also
influence the rate of gastric emptying by comparing plasma glucose and gastrointestinal hormone responses to an oral **sucrose** load with and without acarbose in 11 healthy subjects. Gastric emptying was assessed indirectly by measuring
circulating paracetamol concentrations following administration of paracetamol along with the **sucrose** load. Peak plasma glucose, insulin, and glucose-dependent insulinotropic **polypeptide** (GIP) responses were reduced when
sucrose was given with acarbose. There was a significant reduction in post-sucrose paracetamol levels with acarbose suggestive of a significant delay in gastric emptying. The failure of acarbose to induce change in
circulating paracetamol concentrations until after 60 min is indicative of a delay in gastric emptying rather than an osmotic malabsorption. The exaggerated and **sustained release** of glucagon-like **peptide**-1
(7-36)amide (GLP-1) seen when **sucrose** was given with acarbose may play a part in the inhibition of gastric emptying. This study indicates that a significant delay in gastric emptying may be an added mechanism contributing to the
therapeutic effect of acarbose.

L14 ANSWER 27 OF 31 TOXCENTER COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2002:140896 TOXCENTER
COPYRIGHT: Copyright 2005 ACS
DOCUMENT NUMBER: CA12610135500D
TITLE: In vitro evaluation of Poly(DL-lactide-co-glycolide) polymer-based implants containing the α-melanocyte stimulating hormone analog, Melanotan-I
AUTHOR(S): Bhardwaj, Renu; Blanchard, James
CORPORATE SOURCE: Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ, USA.
SOURCE: Journal of Controlled Release, (1997) Vol. 45, No. 1, pp. 49-55.
CODEN: JCREEC. ISSN: 0168-3659.
COUNTRY: UNITED STATES
DOCUMENT TYPE: Journal
FILE SEGMENT: CAPLUS
OTHER SOURCE: CAPLUS 1996:737322
LANGUAGE: English
ENTRY DATE: Entered STN: 20020626
Last Updated on STN: 20020626

TI In vitro evaluation of Poly(DL-lactide-co-glycolide) polymer-based implants containing the α-melanocyte stimulating hormone analog, Melanotan-I

AB The release of the melanotropic **peptide**, Melanotan-I (MT-I), from biodegradable implants of poly(DL-lactide-co-glycolide) (PLGA) copolymer was studied. The implants were prepared by a melt-extrusion method. The in vitro release of

MT-I exhibited a triphasic profile with an initial rapid release followed by a secondary phase of **slow release**, then a tertiary phase of rapid release due to erosion of the polymer. The initial rapid release observed with PLGA

(50:50) was <5% of the drug load and the tertiary phase commenced after about 3 wk. The factors controlling the drug release are degradation and erosion of the polymer which may, in turn, be controlled by the phys. properties of the polymer such

as mol. weight and viscosity. The influence of viscosity (0.2-1.08 dL/g) of the polymer, on the release kinetics of MT-I were analyzed and the polymer having a viscosity of 0.6 dL/g was selected for preparing a 1-mo implant system. Mol. weight

distribution anal. indicated a biphasic rate of mol. weight reduction and within 12 days, the mol. weight had decreased to 50% of the initial value. The release rate was examined at different drug loading levels and in the presence of some hydrophilic

additives. The effect of γ-irradiation on the release kinetics of the **peptide** was analyzed to determine the optimal radiation sterilization dose for the PLGA implants. There was no significant difference in the total duration of MT-I

release between the implants exposed to no radiation and the 2.5 Mrad dose selected.

L14 ANSWER 28 OF 31 TOXCENTER COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER:	1996:193497 TOXCENTER
COPYRIGHT:	Copyright 2005 ACS
DOCUMENT NUMBER:	CA12514177462C
TITLE:	Surface-modified nanoparticles and method of making and using them
AUTHOR(S):	Levy, Robert J.; Labhasetwar, Vinod; Song, Cunxian S.
PATENT INFORMATION:	WO 9620698 A2 11 Jul 1996
SOURCE:	(1996) PCT Int. Appl., 170 pp. CODEN: PIXXD2.
COUNTRY:	UNITED STATES
DOCUMENT TYPE:	Patent
FILE SEGMENT:	CAPLUS
OTHER SOURCE:	CAPLUS 1996:544101
LANGUAGE:	English
ENTRY DATE:	Entered STN: 20011116 Last Updated on STN: 20020730

TI Surface-modified nanoparticles and method of making and using them

AB Biodegradable controlled-release nanoparticles as **sustained release** bioactive agent delivery vehicles include surface modifying agents to target binding of the nanoparticles to tissues or cells of living systems, to enhance

nanoparticle **sustained release** properties, and to protect nanoparticle-incorporated bioactive agents. Unique methods of making small (10 nm to 15 nm, and preferably 20 nm to 35 nm) nanoparticles having a narrow size

distribution which can be surface-modified after the nanoparticles are formed is described. Techniques for modifying the surface include a lyophilization technique to produce a phys. adsorbed coating and epoxy-derivatization to functionalize

the surface of the nanoparticles to covalently bind mols. of interest. The nanoparticles may also comprise hydroxy-terminated or epoxide-terminated and/or activated multiblock copolymers, having hydrophobic segments which may be

polycaprolactone and hydrophilic segments. The nanoparticles are useful for local intravascular administration of smooth muscle inhibitors and antithrombogenic agents as part of interventional cardiac or vascular catheterization such as a

balloon angioplasty procedure; direct application to tissues and/or cells for gene therapy, such as the delivery of osteotropic genes or gene segments into bone progenitor cells; or oral administration in an enteric capsule for delivery of

protein/**peptide** based vaccines.

L14 ANSWER 29 OF 31 TOXCENTER COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1994:153379 TOXCENTER
COPYRIGHT: Copyright 2005 ACS
DOCUMENT NUMBER: CA12102018033Q
TITLE: Preparation of biodegradable polycarbonates, and their use as drug carriers
AUTHOR(S): Acemoglu, Murat
CORPORATE SOURCE: ASSIGNEE: Sandoz Ltd.
PATENT INFORMATION: WO 9320126 A1 14 Oct 1993
SOURCE: (1993) PCT Int. Appl., 73 pp.
CODEN: PIXXD2.
COUNTRY: AUSTRIA
DOCUMENT TYPE: Patent
FILE SEGMENT: CAPLUS
OTHER SOURCE: CAPLUS 1994:418033
LANGUAGE: English
ENTRY DATE: Entered STN: 20011116
Last Updated on STN: 20040622

TI Preparation of biodegradable polycarbonates, and their use as drug carriers
AB A biodegradable and biocompatible polycarbonate is disclosed, comprising C3-10 alkylene carbonic acid ester units, each alkylene group being a C3-alkylene with 1 oxy substituent or a C4-10 alkylene with 2-8 oxy substituents, each of the oxy substituents occurring individually as a hydroxyl group or as a derivatized hydroxyl group comprising an ester or ortho ester or acetal residue. The polycarbonates may be used as matrixes for the **sustained release** of pharmacol. active compds., e.g. **peptides** or proteins, in the form of microparticles or implants. Preparation of the polymers of the invention is included, as are degradation kinetics and drug release from an implant containing a polymer of the invention.

L14 ANSWER 30 OF 31 BIOTECHNO COPYRIGHT 2005 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1993:23134189 BIOTECHNO
TITLE: Controlled release of **polypeptides** from polyanhydrides
AUTHOR: Ron E.; Turek T.; Mathiowitz E.; Chasin M.; Hageman M.; Langer R.
CORPORATE SOURCE: Department of Chemical Engineering, Massachusetts Technology Institute,Cambridge, MA 02138, United States.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1993), 90/9 (4176-4180)
CODEN: PNASA6 ISSN: 0027-8424
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English

TI Controlled release of **polypeptides** from polyanhydrides
AN 1993:23134189 BIOTECHNO
AB The effects of both polymer hydrophobicity and addition of stabilizers on the release and integrity of polymer-encapsulated proteins were studied. By using very hydrophobic poly¢1,3-bis(p-carboxyhydroxy)hexane anhydride! with **sucrose** as an excipient, both recombinant bovine somatotropin and zinc insulin were released intact over 3 weeks. The released proteins appeared to maintain their integrity as judged by acidic reverse-phase HPLC, size- exclusion HPLC, radioimmunoassay, and conformation-sensitive immunoassays. Our results also suggest how polymer hydrophobicity can be used to enhance protein stability.

L14 ANSWER 31 OF 31 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
ACCESSION NUMBER: 1993:78015 BIOSIS
DOCUMENT NUMBER: PREV199395042515
TITLE: Gonadotropin-releasing hormone antagonists with N-omega-triazolylornithine, -lysine, or p-aminophenylalanine residues at positions 5 and 6.

AUTHOR(S): Rivier, Jean [Reprint author]; Porter, John; Hoeger, Carl; Theobald, Paula; Craig, A. Grey; Dykert, John; Corrigan, Anne; Perrin, Marilyn; Hook, William A.
CORPORATE SOURCE: Salk Inst. Biol. Studies, 10010 North Torrey Pines Rd., La Jolla, Calif. 92037, USA
SOURCE: Journal of Medicinal Chemistry, (1992) Vol. 35, No. 23, pp. 4270-4278.
CODEN: JMCMAR. ISSN: 0022-2623.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 26 Jan 1993
Last Updated on STN: 17 Mar 1993

TI Gonadotropin-releasing hormone antagonists with N-omega-triazolylornithine, -lysine, or p-aminophenylalanine residues at positions 5 and 6.

AB In order to be used as fertility regulators in humans, gonadotropin releasing hormone (GnRH) antagonists must be extremely potent and long acting and exhibit negligible side effects such as stimulating histamine release. To this aim, we have recently synthesized a series of analogues with the standard Ac-DNal-1-DCpa-2-DPal-3 substitutions, where the N-omega-amino function of ornithine, lysine, or p-aminophenylalanine (Aph) was converted to the aminotriazolyl (atz) derivatives at positions 5 and 6 with further modifications at positions 7 and 10. The analogues were tested for their ability to bind to pituitary cell membranes, to release histamine in a mast cell assay, to inhibit luteinizing hormone (LH) secretion by castrated male rats or cultured pituitary cells, and to interfere with the ovulation in intact female rats. While the subcutaneous (sc) injections of 50 mu-g of Azaline A (7, (Ac-DNal-1-,DCpa-2,DPal-3,Lys-5(atz),DLys-6,(atz),ILys-8,DAla-10)GnRH) dissolved in 0.2 mL of an aqueous media significantly inhibited LH releases in the castrated male rat for 24 h, the same dose of Azaline B (11), (Ac-DNal-1,DCpa-2,DPal-3,Aph-5(Atz),DAph-6(atz),ILys-8, DAla-10)GNRH, inhibited LH release for 72 h. A similar long duration of action was observed for Antide ((Ac-DNal-1,DCpa-2,DPal-3,Lys-5(Nic),DLys-6(Nic),ILys-8,DAla-10)GnRH) but not for Nal-Glu ((Ac-DNal-1,DCpa-2,DPal-3,Arg-5,4-(p-methoxybenzoyl)-D-2-Abu-6,DAla-10)GnRH). In the same paradigm, a 5-fold dilution of the **peptide** (50 mu-g in 1 mL) and the use of three injection sites rather than one resulted in significantly shorter duration of action for most of the **peptides** tested. This suggested that long duration of action might be the result of **slow release** from the injection site(s). In order to investigate this possibility, Nal-Glu and Azaline B were injected intravenously (iv) at three doses (10, 50, 250 mu-g) to castrated male rats. At all doses, both **peptides** significantly lowered LH levels for 8 h. By 24 h, Nal-Glu (250 mu-g) and Azaline B (50 and 250 mu-g) still measurably inhibited LH secretion. Finally, only Azaline B (250 mu-g) was still active at 48 h. These findings demonstrate that subtle structural modifications will yield **peptides** with different half-lives after iv administration. These findings led us to investigate the effects of other structural modifications on duration of action. We observed that systematic substitutions at positions 7 (NMeLeu) and 10 (Pro-9-NHEt, and Gly-NH-2) were found to be deleterious. Of interest was the observation that only the DAla-10-NH-2 substitution led to long duration of action and enzymatic stability under the conditions tested. Most analogues (excluding (Ac-DNal-1,DCpa-2,DPal-3,DCit-6,DAla-10)GnRH (SB-75), (Ac-DNal-1,DCpa-2,DPal-3,DHar-6(N-g,N-g'-Et-2),Har-8(N-g,N-g'-Et-2),DAla-10)GnRH (Rs-26306) and ((Ac-DNal-1,DCpa-2,DPal-3,NMeTyr-5,DLys-6(Nic),ILys-8,DAla-10)GnRH (A-75998) which were recently reported to be long acting) were tested for binding affinity to pituitary cell membranes. On the basis of the limited data obtained

in that assay, a correlation may exist between nonparallelism (with the standard (DAla-6,NMeLeu-7,Pro-9-NHEt)GnRH) in the dose-reponse curve and long duration of action. In an in vitro histamine release assay and the rat antiovulatory assay

(AOA), Azaline B (ED-50 = 224 +- 23 mu-g/mL: AOA ED-100 = 1.0 mu-g) compared favorably against Nal-Glu (ED-50 = 1.8 +- 0.66 mu-g/mL: AOA ED-100 = 1.5 mu-g), SB-75 (ED-50 = 2.1 +- 0.3 mu-g/ mmL: AOA ED-100 = 2.0 mu-g), RS-26306 (ED-50 = 11 +- 1.1 mu-g/mL: AOA ED-100 gtoreq 2.5 mu-g) or A-75998 (ED-50 = 22 +- 3.2 mu-g/mL: AOA ED-100 mchgt 1.0 mu-g). Azaline B is readily soluble in water/3% mannitol/5% ethanol (gtoreq 20 mg/mL) and is readily produced synthetically.

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